Supplement to SHOCK Vol. 13, 2000

SHOCK®

Injury, Inflammation, and Sepsis: Laboratory and Clinical Approaches

Abstracts
Twenty-Third Annual Conference on Shock
Snowbird, Utah

Saturday, June 3 to Tuesday, June 6, 2000

The Official Journal of

The Shock Society
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The International Federation of Shock Societies, and
The Official and International Journal of the Japan Shock Society

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Summary of Symposia at the Twenty-Third Annual Conference on Shock held in Snowbird, Utah June 3-6, 2000
Summary of Symposia at the 8th International Cytokine Conference held in Amsterdam, November 5-9, 2000.
Both Symposia were very successful. The attached Summaries describe the state-of-the-ART scientific progress being made in these vital areas. Also attached are abstracts of all the papers delivered at the Conference.

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February 19, 2001

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RE: Grant N00014-00-1-0708

Dear Dr. Majde,

On behalf of the Scientific Program Committee, Officers and Council of the Society, I want to thank the Office of Naval Research for the Support of Symposia and Awards at the Twenty-Third Annual Conference on Shock, June 3-9, 2000, Snowbird, Utah and the Eighth International Cytokine Conference, November 5-9, 2000, RAI, Amsterdam.

These meetings were very successful and attended by scientists and physicians throughout the world. We are grateful for the support of the Department of the Navy which helped make this meeting possible.

I am enclosing a copy of *SHOCK* Volume 13, 2000 supplement which contains the program and abstracts (pages 1- 68) for the Shock Conference. A summary of the meeting is also enclosed.

I am enclosing a copy of *EUROPEAN CYTOKINE NETWORK* Volume 11, November, 2000, which contains the abstracts (pages 8-236) for the Cytokine Conference. A summary of the conference is also enclosed.

I look forward to a continued association of the Society with the Office of Naval Research.

Sincerely,

Sherwood M. Reichard
Executive Director

Enclosure

SHOCK SOCIETY
TWENTY-THIRD ANNUAL CONFERENCE ON SHOCK

SNOWBIRD, UTAH
June 3-6, 2000

REPORT

The Twenty-Third Annual Conference on Shock was held in the spectacular mountain resort of Snowbird, Utah. The setting was outstanding. The scientific program consisted of symposia, plenary sessions, workshops and poster sessions. Following are symposia and workshops along with the summary of each session.

SYMPOSIA

Signal Transduction and Genetic Regulation of inflammation
Moderator: **Timothy Buchman**, MD, PhD, Washington University School of Medicine,
St. Louis, Missouri

In this session, the speakers explored the regulatory responses to inflamation with a focus on balance among intracellular pathways. Dr. DeMaio spoke on the diversity of responses and addressed genotype-phenotype relationships. He described approaches to the identification of genes which modulate the inflammatory response. Dr. Giroir focused on TNF-alpha and the consequences of having too much, or too little of this signaling molecule at the surface of a cell. Dr. Callery described how binding of specific ligands changed the state of second messenger intermediates a systematic and reciprocally regulated mechanism. Dr. Moldawer discussed disruption of regulatory responses using gene therapy as an investigative tool. Dr. Cobb compared and contrasted the reductionist analysis of single gene responses with the connectionist analysis afforded by gene arrays("chips") in the study of inflammatory responses. This session emphasized networks over pathways and focused on the idea that the state of biological networks is regulated by competing stimuli and not by isolated signals.

Neuro Endocrine Interaction: Regulation of Responses to Shock and Trauma
Moderator: **Naji N. Abumrad,** MD, North Shore University Hospital, Manhasset, New York.

Understanding the control mechanisms involved in modulation of the hemodynamic, pro-inflammatory, metabolic and immune responses which occur during the ebb and flow phases following injury is crucial in order to establish optimal intervention paradigms for the critically ill individual. Studies using various models of physical stress, including hemorrhagic and endotoxic shock, trauma and hypoclycemia, have provided significant evidence of a critical role for neuro-endocrine control of these responses. The pathways involved in modulation of the magnitude and time course of these post-traumatic stress responses are not limited to hypothalamo-pituitary-axis activation, but include central and peripheral release of opioids, excitatory amino acids, serotonin and nitric oxide. These neuro-endocrine mediators play redundant adjuvant or opposing roles affecting the wide array of immune, metabolic and hemodynamic responses, which comprise the post-injury phase. The aim of this symposium was to highlight some of the recent advances in the understanding of neuro-endocrine control of select responses to shock, trauma and sepsis.

Understanding of Myocardial Dysfuction in Hyper Inflammatory States
Moderator: **Kathleen McDonough**, PhD, Louisiana State University, New Orleans

The Myocardium is responsible for pumping a cardiac output to match the tissue's requirements for blood flow. Alterations in myocardial function are normally elicited by changes in preload, afterload, contractility and heart rate. However, during inflammatory states, sepsis and compromised myocardial blood flow, changes in myocardial contractile function can occur through other influences such as acidemia, cytokines and chemokines, oxygen radicals and a number of other mediators that may be produced in an inflammatory state. The aim of this symposium was to present an update of the intracellular mechanisms by which myocardial contractile function is depressed and the role of cytokines in this myocardial depression. In response to injury, the myocardium can upregulate protective functions that serve to blunt the negative consequences of a second insult to the heart. Mechanisms involved in inducing cardioprotection, including the potential role of cytokines, were discussed. Methods to assess and treat myocardial dysfunction in the clinical setting were presented. Finally, the issues of potential mechanisms of injury versus mechanisms that have actually been shown to contribute to dysfucntion in a pathophysiological state were discussed.

WORKSHOPS

Understanding SIRS and MOF: Time to Change Perspective
Moderator: **Gill Cryer**, MD, PhD, University of California, Los Angeles

Multiple Organ Failure remains one of the most common causes of death after injury or sepsis. Despite incredible advances in critical care technology over the last 30-40 years the mortality rate for this syndrome remains very high. There have been tremendous gains in our knowledge from basic research, yet this knowledge has not resulted in significant improvements in outcome in the clinical setting. Perhaps we need to look at the problem differently. In this symposium it was attempted to look at the problem of Multiple Organ Failure from different perspectives. Hopefully new ideas were generated, which may eventually lead to improved outcomes for patients suffering from this disease.

Recent Adjuncts to Resuscitation Strategies to Prevent the SIRS to MOF Progression: Bench to Bedside
Moderator: **Kenneth Proctor**, PhD, University of Tennessee, Memphis

After severe trauma and blood loss, aggressive fluid resuscitation may be the only hope for saving the patient. At the same time, reperfusion promotes reactive oxygen metabolite generation and activates PMNs in splanchnic and other tissues that are already expressing multiple cytokine and endothelial cell surface adhesion molecules. The resultant hyper-inflammatory state can produce secondary injury locally in otherwise undamaged cells, can spill over into remote organs (e.g. lung), or can propagate into a malignant unregulated systemic response leading to SIRS or MOF. One speaker described clinically-relevant models of battlefield injuries designed to mimic these conditions. The second speaker described the benefits of the novel blood substitute in urban trauma patients, compared to other resuscitation fluids and compared to potential transfusion-induced cytotoxicity cased by stored, packed RBCs. The third speaker considered novel strategies in the critically ill trauma patient that combine adequate cellular resuscitation and avoidance of splanchnic vasopressors. Such strategies prevent or ameliorate the ravages of unfettered oxidative stress using agents that attenuate or block unregulated cytotoxin formation and "unprime" PMNs and are initiated in the trauma resusucitation area, ER, or surgical OR. The final speaker provided an updated review on a number of the clinical trials of new theraputic agents for the adjuvant treatment of shock, sepsis, and/or SIRS which have just closed to enrollment, are in progress, or are in the final planning stages.

INTERNATIONAL CYTOKINE SOCIETY
EIGHTH INTERNATIONAL CYTOKINE CONFERENCE

RAI, Amsterdam
November 5-9, 2000

Report

The Eighth International Cytokine Conference was held in RAI, Amsterdam together with the International Society for Interferon and Cytokine Research (ISICR)

The program consisted of 12 Symposia, 21 workshops and 9 review lectures. The topics covered are listed below:

Cytokines and T cell differentiation

Cytokines in sepsis and toxic shock

Cytokine/chemokines in allergy

Cytokine and interferon gene regulation I

New/second generation interferons and cytokines I

Suppressors of cytokine signaling (SOCS)

Cytokine and interferon gene regulation II

New/second generation interferons and cytokines II

Receptor-ligand interactions

Signal transduction I

Clinical use of cytokines and interferons

Functional polymorphism of cytokine genes

Signal transduction II

Type I interferons: Selective signaling and effects on the nervous system

Cytokines and interferons in hemopoiesis and angiogenesis

Interferon-inducible proteins (includes PKR)

Cytokine-binding proteins

Immunosuppressive cytokines

The renaissance of IFN-B including its effect on MS and EAE Chemokines

Genomic structure and function of interferon and cytokine genes

Cytokines in neurological disease (includes MS and EAE)

Regulation of cytokine and interferon mRNA stability

Cyokines and interferons in transplantation

Mode of action of cytokines I

Signal transduction II

Oral/nasal interferons and cytokines

Mode of action of interferons

Cytokines and interferons in cancer

Chemokines, HIV and vaccine

Interferons and cytokines in infectious disease I

Mode of action of cytokines II

Cytokines and interferons in autoimmunity

Toll and apoptosis

Interferons and cytokines in infectious disease II

Viral anticytokine strategies

This meeting is dedicated to the memory of:

William Schumer

1926–2000

Founding President
1978–1979

Editor, Circulatory Shock
1980–1987

Bill Schumer's influence on this Society was profound. He helped establish the Shock Society in 1977, was the first President in 1978 and Program Chair of the first national meeting at the Airlie Conference Center, Airlie, Virginia June 1–3, 1978. His choice of Airlie set the tone for venues for the Shock Society ever since; beautiful places where shock researchers of both clinical and basic sciences could meet together and set the stage for long lasting collaborative relationships.

Bill took over the editorship of the Journal in 1980 and continued working to integrate the clinical and basic sciences and continued his quest for excellence. He took the journal, which now became the Official Journal of the Shock Society, to new heights during his eight years as Editor.

Bill came to every meeting and played an active role. He especially liked the workshops where his active participation and personal commitment made him a wonderful role model for students.

Bill Schumer will be missed not only for his seminal work on cellular metabolism in shock, pathophysiology of septic shock and the role of glucocorticoids as therapeutic agents in septic shock, but his vision, energy and enthusiasm which served to mold this great Society will also be his legacy into the future.

Twenty-Third Annual Conference on Shock
Snowbird, Utah

Saturday, June 3 to Tuesday, June 6, 2000

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Kathleen McDonough, PhD, 1996-1998
Carol L. Wells, PhD, 1998-2000

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1st June 1-3, 1978, Airlie, Virginia
William Schumer, MD, Chair
Abstracts: Circulatory Shock 5:2, 182-232, 1978
Papers: Advances in Shock Research, Vols. 1 & 2, 1979, and Metabolic and Cardiac Alterations in Shock and Trauma. Circulatory Shock Supplement 1, 1979

2nd June 7-9, 1979, Williamsburg, Virginia
David G. Reynolds, PhD, Chair
Abstracts: Circulatory Shock 6:2, 165-198, 1979
Papers: Advances in Shock Research, Vols. 3 & 4, 1980

3rd June 11-13, 1980, Lake of the Ozarks, Missouri
Lerner B. Hinshaw, PhD, Chair
Abstracts: Circulatory Shock 7:2, 187-223, 1980
Papers: Advances in Shock Research, Vols. 5 & 6, 1981

4th June 4-6, 1981, Marco Island, Florida
Sherwood M. Reichard, PhD, Chair
Abstracts: Circulatory Shock 8:2, 1981
Papers: Advances in Shock Research, Vols. 7 & 8, 1982

5th June 9-11, 1982, Smuggler's Notch, Vermont
Robert R. Wolfe, PhD, Chair
Abstracts: Circulatory Shock 9:2, 1982
Papers: Advances in Shock Research, Vols. 9 & 10, 1983

6th June 6-8, 1983, Grand Teton National Park, Wyoming
Robert W. Phillips, PhD, Chair
Abstracts: Circulatory Shock 10:3, 1983

7th June 4-6, 1984, Toronto, Canada
Glen A. Taylor, MD, Chair
Abstracts: Circulatory Shock 13:1, 1984

8th June 9-12, 1985, Baltimore, Maryland
Daniel L. Traber, PhD, Chair
Abstracts: Circulatory Shock 16:1, 1985

9th June 8-11, 1986, Scottsdale, Arizona
Gerald S. Moss, MD, Chair
Abstracts: Circulatory Shock 18:4, 1986

10th June 7-11, 1987, Montreal, Canada
Robert F. Bond, PhD, Chair
Abstracts: Circulatory Shock 21:4, 1987

11th June 5-8, 1988, Lake Geneva, Wisconsin
John C. Passmore, PhD, Chair
Abstracts: Circulatory Shock 24:4, 1988

12th June 9-12, 1989, Marco Island, Florida
Irshad H. Chaudry, PhD, Chair
Abstracts: Circulatory Shock 27:4, 1989

13th June 8-11, 1990, Durango, Colorado
H. Richard Adams, DVM, PhD, Chair
Abstracts: Circulatory Shock 31:1, 1990

14th June 2-6, 1991, Vienna, Austria
John W. Holaday, PhD, Chair
Abstracts: Circulatory Shock 34:1, 1991

15th June 7-10, 1992, Point Clear, Alabama
Donald E. Fry, MD, Chair
Abstracts: Circulatory Shock 37:1, 1992

16th June 13-16, 1993, Santa Fe, New Mexico
James A. Cook, PhD, Chair
Abstracts: Circulatory Shock Supplement 2, 1993

17th June 5-8, 1994, Big Sky, Montana
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Abstracts: SHOCK Supplement 1, 1994

18th June 11-14, 1995, Asheville, North Carolina
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Abstracts: SHOCK Supplement 2, 1995

19th June 2-5, 1996, Grand Traverse, Michigan
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20th June 15-18, 1997, Indian Wells, California
Edwin A. Deitch, MD, Chair
Abstracts: SHOCK Supplement 2, 1997

21st June 14-17, 1998, San Antonio, Texas
Mark G. Clemens, PhD, Chair
Abstracts: SHOCK Supplement 1, 1998

22nd June 12-16, 1999, Philadelphia, Pennsylvania
Allan M. Lefer, PhD, Chair
Abstracts: SHOCK Supplement 1, 1999

23rd June 3-6, 2000, Snowbird, Utah
H. Gill Cryer, MD, PhD, Chair
Abstracts: SHOCK, Supplement 2, 2000

International Congresses

1st June 7-11, 1987, Montreal, Canada
Robert F. Bond, PhD, Chair
Abstracts: Circulatory Shock 21:4, 1987

2nd June 2-6, 1991, Vienna, Austria
Gunther Schlag, MD, Chair
Abstracts: Circulatory Shock 34:1, 1991

3rd Third International Shock Congress
Kazuo Okada, MD, Chair
Act City Hamamatsu, Japan
October 21-23, 1995
Abstracts: SHOCK Supplement 3, 1995

4th Fourth International Shock Congress
Allan M. Lefer, PhD, Chair
June 12-16, 1999
Abstracts: SHOCK Supplement 1, 1999

International Symposia

July 17-24, 1980, Budapest, Hungary
Cosponsors: Shock Society and International Congress of Physiology, Arisztid G.B. Kovách, John J. Spitzer, and H. B. Stoner, Chairs, Papers: Advances in Physiological Sciences, Vol. 26, Homeostasis in Injury and Shock, Pergamon Press, 1981

September 5-8, 1984, Manchester, England
"The Scientific Basis of the Care of the Critically Ill"
M.H. Irving and R.A. Little, Chairs
Partially supported by the Shock Society

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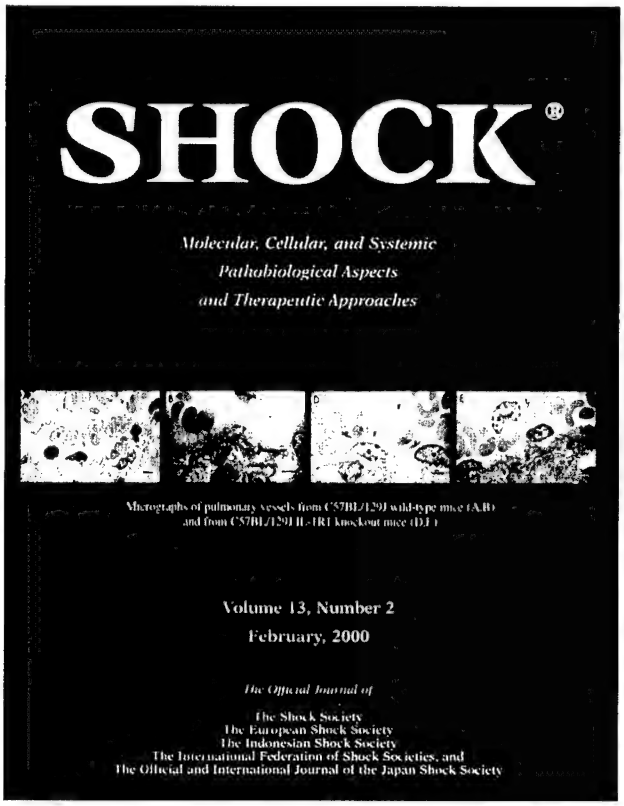

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SHOCK SOCIETY
TWENTY- THIRD ANNUAL CONFERENCE ON SHOCK

SNOWBIRD, UTAH
June 3-6, 2000

SATURDAY, JUNE 3, 2000

9:00 - 2:00 PM **COUNCIL MEETING**
White Pine

1:00 - 6:00 PM **Registration**
Ballroom Foyer

2:00 - 3:00 PM **PENARY SESSION I**, Papers 1-5
Ballroom 1 & 2 Moderators: **Edwin A. Deitch, MD** , UMDNJ-New Jersey Medical School, Newark and **Gregory Bagby, MD**, Louisiana State University Medical Center, New Orleans

2:00 pm **Post-Hemorrhagic Shock Mesenteric Lymph (PHSML) Lipids Prime Neutrophil Superoxide Production Via Phospholipase A2**, Paper 1
Ricardo J. Gonzalez, MD
Denver Health Medical Center, Denver, Colorado

2:12 pm **Non-Compartmentalization of Granulocyte-Colony Stimulating Factor (G-CSF) Following an Intrapulmonary Bacterial Challenge**, Paper 2
Gregory J. Bagby, MD
Louisiana State University Medical Center, New Orleans

2:24 pm **Glucose-6-P Dehydrogenase (G6PD) Deficiency Predisposes to Sepsis, Worsens Anemia and Results in a Pronounced Activation of Circulating Monocytes After Severe Trauma**, Paper 3
Zoltan Spolarics, MD, PhD
UMDNJ- New Jersey Medical School, Newark

2:36 pm **Burn Injury Induces Expression of Two Novel Forms of the TIS11D Gene in Mice**, Paper 4
Kristina G. Hobson, MD
Shriners Hospitals for Children, Sacramento, California

2:48 pm **The Role of INF-γ and IL-12 on Propioni-Bacterium (PA) Acnes-Primed LPS Hepatic Injury**, Paper 5
Yoshiaki Shimizu, MD
Washington University, St. Louis, Missouri

3:00-5:00PM **WORKSHOP I: Understanding SIRS and MOF: Time to Change Perspective**
Ballroom 1 & 2 Moderator: **Gill Cryer, MD, PhD,** University of California, Los Angeles

Multiple Organ Failure remains one of the most common causes of death after injury or sepsis. Despite incredible advances in critical care technology over the last 30-40 years the mortality rate for this syndrome remains very high. There have been tremendous gains in our knowledge from basic research, yet this knowledge has not resulted in significant improvements in outcome in the clinical setting. Perhaps we need to look at the problem differently. In this symposium we will attempt to look at the problem of Multiple Organ Failure from different perspectives. Hopefully we will generate new ideas, which may eventually lead to improved outcomes for patients suffering from this disease.

Saturday Continued

3:00 pm	**Introduction** Gill Cryer, MD, PhD
3:10 pm	**Recent Advances from Basic Research Tells Us It's Time to Change the Definitions of SIRS and MOF** Edward Abraham, MD, University of Colorado Health Sciences Center, Denver
3:35 pm	**The Difference Between MOF and SIRS is Really a Failure of Recovery** Timothy Buchman, MD, PhD, Washington University School of Medicine, St. Louis, Missouri
4:00 pm	**The SIRS-MOF Continuum is a Failed Dynamic Balancing Act Across Time** John Mannick, MD, Harvard Medical School, Boston, Massachusetts
4:25 pm	**The Solution to SIRS and MOF: A Challenge for the New Millennium** Eugen Faist, MD, Ludwig-Maximilians-Universität, München Germany

7:30-8:30PM
Ballroom 1 & 2

KEYNOTE ADDRESS: Oh, the Places You'll Go: Will You Succeed? You Will Indeed! 99 and 3/4 Percent Guaranteed
Jureta W. Horton, PhD, President-Elect, University of Texas Southwestern Medical School, Dallas

8:30 - 9:30 PM
Conference Center Terrace

OPENING RECEPTION

SUNDAY, JUNE 4, 2000

7:00 - 8:00 AM
Maybird

EDITORIAL BOARD BREAKFAST

7:00 - 8:00 AM
Ballroom 3, Magpie & Golden Cliff

Continental Breakfast

7:00 - 9:00 AM
Ballroom 3, Magpie & Golden Cliff

POSTER SESSION I, Papers 6-76

Adhesion Molecules, Paper 6
Animal Models, Papers 7-10
Burn/Trauma, Papers 11-18
Cellular/Molecular, Papers 19-21
Cytokines, Papers 22-30
Eicosanoids/PAF, Paper 31
Endotoxin/Sepsis, Papers 32-48
Gene Regulation, Paper 49
Immunologic Dysfunction, Paper 50
Immunomodulation, Papers 51-55
Inflammation, Papers 56-58
Metabolism, Papers 59-60
Microcirculation, Papers 61-66
Monocytes/Macrophages, Paper 67
Multiple Organ Failure, Papers 68-69
Myocardial Function, Papers 70-72
Neonatology, Paper 73
Pulmonary, Paper 74
Renal, Papers 75-76

Sunday Continued

9:00 - 10:00 AM
Ballroom 1 & 2

PLENARY SESSION II, Papers 77-81
Moderator: **William Cheadle, PhD**, Veterans Administration Medical Center, Louisville, Kentucky and **Mark Carlson, PhD**, Veterans Administration Medical Center, Omaha, Nebraska

9:00 am — **LPS-Induced, Imbalanced Expression of Hepatic Vascular Stress Genes in Cirrhosis: Mechanism of Increased Susceptibility to Endotoxemia**, Paper 77
Rajiv Baveja
University of North Carolina, Charlotte

9:12 am — **Role of Nitric Oxide in Hemorrhagic Shock-Induced Hepatic Heme Oxygenase-1 Expression in the Rat**, Paper 78
Alexander Hoetzel, MD
University of Freiburg, Germany

9:24 am — **Interleukin (IL)-6 Knockout Attenuates Early Sepsis-Associated Hepatic Gene Downregulation but Increases Hepatic Necrosis and Death**, Paper 79
Patrick K. Kim, MD
University of Pennsylvania, Philadelphia

9:36 am — **A Dominant Role of P55 TNF-α Receptor in Endotoxemic Myocardial Dysfunction**, Paper 80
Xianzhong Meng
University of Colorado Health Sciences Center, Denver

9:48 am — **Evidence for a Role of NF-κB in Acute Hypovolemic Hemorrhagic Shock in Rats**, Paper 81
Francesco Squadrito, MD
University of Messina, Italy

10:00 AM - 12:00 PM
Ballroom 1 & 2

SYMPOSIUM I: Signal Transduction and Genetic Regulation of Inflammation
Moderator: **Timothy Buchman, MD, PhD**, Washington University School of Medicine, St. Louis, Missouri

In this session, the speakers will explore the regulatory responses to inflammation with a focus on balance among intracellular pathways. Dr. DeMaio will speak on the diversity of responses and address genotype-phenotype relationships. He will describe approaches to the identification of genes which modulate the inflammatory response. Dr. Giroir will focus on TNF-α and the consequences of having too much, or too little of this signalling molecule at the surface of a cell. Dr. Callery will describe how binding of specific ligands changes the state of second messenger intermediates a systematic and reciprocally regulated mechanism. Dr. Moldawer will discuss disruption of regulatory responses using gene therapy as an investigative tool. Dr. Cobb will compare and contrast the reductionist analysis of single gene responses with the connectionist analysis afforded by gene arrays ("chips") in the study of inflammatory responses. This session will emphasize networks over pathways and focus on the idea that the state of biological networks is regulated by competing stimuli and not by isolated signals.

10:00 am — **Genetic Diversity in the Response to Canonical Inflammatory Stimuli**
Antonio DeMaio, PhD, Johns Hopkins University School of Medicine, Baltimore, Maryland

10:24 am — **TNF-α: Inflammation in Moderation**
Brett Giroir, MD, University of Texas Southwestern Medical School, Dallas

Sunday Continued

10:48 am	**Balanced Responses of the Second Messenger Pathways to Inflammation** Mark P. Callery, MD, University of Massachusetts Medical School, Worcester
11:12 am	**Gene Therapy as a Strategy for Modulating the Response to Inflammation** Lyle L. Moldawer, PhD, University of Florida College of Medicine, Gainesville
11:36 am	**Top-Down (Arrays) Versus Bottom-Up (Specific Gene) Approaches to the study of Responses to Inflammation** J. Perren Cobb, MD, PhD, Washington University, St. Louis, Missouri
10:45 - 11:00 AM **Ballroom Foyer**	**Coffee Available**
12:00 - 1:30 PM **Cottonwood 1-4**	**Lunch**
1:45 - 3:15 PM **Ballroom 1 & 2**	**YOUNG INVESTIGATOR AWARD SESSION**, Papers 82-85 Presiding: **Mohammed M. Sayeed, PhD**, Loyola University, Maywood, Illinois
1:45 pm	**Cytokine-Induced Enterocyte-Derived Nitric Oxide Induces Intestinal Monolayer Injury in an Autocrine Fashion**, Paper 82 Raquel M. Forsythe, MD UMDNJ-New Jersey Medical School, Newark
2:00 pm	**Progesterone Improves Cardiovascular Function Following Trauma-Hemorrhage and Resuscitation**, Paper 83 Joachim Friedrich Kuebler, MD Rhode Island Hospital, Providence
2:15 pm	**Do Peripheral Blood Mononuclear Cells Mimic the Sexually Dimorphic Immune Response of Tissue Immune Cells Following Trauma-Hemorrhage?**, Paper 84 Christian P. Schneider, MD Rhode Island Hospital, Providence
2:30 pm	**Glutamine Induces Heat Shock Protein and Prevents Mortality from Endotoxemia in the Rat**, Paper 85 Paul E. Wischmeyer, MD University of Chicago, Illinois
3:00 - 3:30 pm **Ballroom Foyer**	**Coffee Break**
3:30 - 5:30 PM **Ballroom 1 & 2**	**MINISYMPOSIUM I**, Papers 86-95 Moderator: **Alfred Ayala, PhD**, Rhode Island Hospital, Providence and **Inge Bauer, PhD**, University of Saarland, Homburg, Germany
3:30 pm	**Intraluminal Nutrients Enhance Gut Ischemia/Reperfusion Injury**, Paper 86 Rosemary Kozar, MD, PhD University of Texas Medical School, Houston

Sunday Continued

3:42 pm	**Effects of the Secretion of Metabolic Regulating Hormones (Leptin) and Posttraumatic Complications in Blunt Polytrauma Patients**, Paper 87 Martijn van Griensven, PhD Hannover Medical School, Germany
3:54 pm	**Delayed Blockage of FasL Restores Lymphoid Immune Function, Suppresses Apoptosis and Improves Survival in Sepsis**, Paper 88 Chun-Shiang Chung, PhD Rhode Island Hospital, Providence
4:06 pm	**Role of Kupffer Cells and Neutrophils for the Regulation of Heme Oxygenase-1 Gene Expression in the Liver Under Stress Conditions,** Paper 89 Markus Paxian, MD University of Saarland, Homburg, Germany
4:18 pm	**Expression Pattern and Regulation of Heme Oxygenase-1/Heat Shock Protein 32 in Human Liver Cells**, Paper 90 Inge Bauer, PhD University of Saarland, Homburg, Germany
4:30 pm	**Endotoxin Mediated Blockade of Pregnane X Receptor Translocation: Effects on Hepatic Cytochrome P-450**, Paper 91 Clinton Chichester University of Rhode Island College of Pharmacy, Kingston
4:42 pm	**Flagellin, A Novel Mediator of Gram Negative Bacteria-Induced Shock** Paper 92 Andrew L. Salzman, MD Inotek Corporation, Beverly, Massachusetts
4:54 pm	**CD16 Blockade in Polymicrobial Sepsis Increases Hepatic but Not Pulmonary Neutrophil Sequestration**, Paper 93 Stephen A. Rowe, MD Veterans Administration Medical Center, Louisville, Kentucky
5:06 pm	**Adenosine-Mediated Alterations in Testicular Cytokine and Testosterone Production**, Paper 94 Andrew M. Clark, BA University of Illinois at Chicago, Illinois
5:18 pm	**Posttraumatic Disturbances of Humoral Bone Factors in Trauma Patients**, Paper 95 Otmar A. Trentz, MD University Hospital, Zurich, Switzerland

3:30 - 5:30 PM **Superior A & B**	**MINISYMPOSIUM II**, Papers 96-105 Moderators: **Jureta W. Horton, PhD,** University of Texas Southwestern Medical Center, Dallas and **J. Perren Cobb, MD**, Washington University, St. Louis, Missouri
3:30 pm	**Effects of Lactated Ringers on Cardiomyocyte TNF-α Synthesis**, Paper 96 Jureta W. Horton, PhD University of Texas Southwestern Medical Center, Dallas
3:42 pm	**Microvascular Effects of Oral IL-6**, Paper 97 F.M. Rollwagen, PhD Uniformed Services of the Health Sciences, Bethesda, Maryland

Sunday Continued

3:54 pm	**Removal of Fatty Acids Improves Coupling of Ex-Vivo Myocardial Glycolytic Flux to Glucose Oxidation After Hemorrhage**, Paper 98 Lisa T. Thornton Carolinas Medical Center, Charlotte, North Carolina
4:06 pm	**Sepsis Gene Expression Profiling: Murine Splenic Compared to Hepatic Responses Determined Using cDNA Microarrays**, Paper 99 J. Perren Cobb, MD Washington University, St. Louis, Missouri
4:18 pm	**Genetic Disruption of Poly (ADP-Ribose) Synthethase Reduces Gut\ Dysfunction and Distant Organ Damage in Mesenteric Ischemia-Reperfusion Injury**, Paper 100 Lucas Liaudet Inotek Corporation, Beverly, Massachusetts
4:30 pm	**Post Hemorrhagic Shock Mesenteric Lymph Upregulates E-Selectin Expression in Human Umbilical Vein Endothelial Cells (HUVEC)**, Paper 101 Justin T. Sambol, MD UMDNJ-New Jersey Medical School, Newark
4:42 pm	**A Time Course Study of the Protective Effect of Mesenteric Lymph Duct Ligation on Hemorrhagic Shock-Induced Pulmonary Injury and the Toxic Effects of Shock Lymph on HUVEC Cell Monolayer Permeability**, Paper 102 Edwin A. Deitch, MD UMDNJ- New Jersey Medical School, Newark
4:54 pm	**LBP Promotes Bacterial Killing of Silver Sulfadiazine Resistant P. Aeruginosa in Infected Burn Wounds**, Paper 103 Richard D. Klein University of Michigan, Ann Arbor
5:06 pm	**Distribution of Monohydroxy Fatty Acids (MHA) in Murine Skin Following Thermal Injury**, Paper 104 Kenneth Langen Loyola University Medical Center, Maywood, Illinois
5:18 pm	**Which Receptor Mediates Prostaglandin E_2 (PGE_2)/Thromboxane A_2 Synergy?**, Paper 105 F. Mahzari, BS University of Texas Southwestern Medical School, Dallas
6:30 - 7:30 PM **Ballroom Lobby**	**RECEPTION**
7:30 - 9:30 PM **Ballroom 1 & 2**	**DINNER/SPEAKER**

MONDAY, JUNE 5, 2000

6:30 AM **Meet in Lobby**	**Eighteenth Annual Presidential Run**

Monday Continued

8:00 - 9:00 AM **Continental Breakfast**
Ballroom 3, Magpie & Golden Cliff

9:00 - 10:00 AM **PLENARY SESSION III**, Papers 106-110
Ballroom 1 & 2 Moderators: **Ronald V. Maier, MD**, Harborview Medical Center, Seattle, Washington and **Lyle Moldawer, PhD**, University of Florida College of Medicine, Gainesville

9:00 am **Mitogen-Activated Protein Kinases (MAPK) in the ICU: Potential Prognostic Factors**, Paper 106
Matthew R. Rosengart, MD
Harborview Medical Center, Seattle, Washington

9:12 am **Protegrin-1 Enhances Bacterial Killing in Thermally Injured Murine Epidermis**, Paper 107
Lars Steinstresser
University of Michigan, Ann Arbor

9:24 am **Burn-Induced T Cell Suppression is Prevented After Neutrophil Depletion in Burn-Injured Rats**, Paper 108
Thyyar M. Ravindranath
Loyola University Medical Center, Maywood, Illinois

9:36 am **STAT 5/6 Protein and Cytokine Expression,** Paper 109
Vicky Chappell, MD
University of Texas Medical Branch, Galveston

9:48 am **The Inflammatory Response in Severely Injured Patients Following Small Volume Resuscitation**, Paper 110
U.C. Liener, MD
University of Ulm, Germany

10:00AM-12:00PM **SYMPOSIUM II: Neuro Endocrine Interaction: Regulation of Responses to Shock and Trauma**
Ballroom 1 & 2 Moderator: **Naji N. Abumrad, MD**, North Shore University Hospital, Manhasset, New York

Understanding the control mechanisms involved in modulation of the hemodynamic, pro-inflammatory, metabolic and immune responses which occur during the ebb and flow phases following injury is crucial in order to establish optimal intervention paradigms for the critically ill individual. Studies using various models of physical stress, including hemorrhagic and endotoxic shock, trauma and hypoclycemia, have provided significant evidence of a critical role for neuro-endocrine control of these responses. The pathways involved in modulation of the magnitude and time course of these post-traumatic stress responses are not limited to hypothalamo-pituitary-axis activation, but include central and peripheral release of opioids, excitatory amino acids, serotonin and nitric oxide. These neuro-endocrine mediators play redundant, adjuvant or opposing roles affecting the wide array of immune, metabolic and hemodynamic responses, which comprise the post-injury phase. The aim of this symposium is to highlight some of the recent advances in the understanding of neuro-endocrine control of select responses to shock, trauma and sepsis.

10:00 am **Introduction**
Naji N. Abumrad, MD

Monday Continued

10:12 am	**Modulation of Trauma/Shock-Induced Responses; Interaction of Monoamine and Opiate Pathways** Patricia E. Molina, MD, PhD, Louisiana State University Health Science Center, New Orleans
10:39 am	**The Stress/Septic Response: The Role of IGF and Growth Hormone** Charles Lang, PhD, Penn State College of Medicine, Hershey
11:06 am	**The Role of Central Parasympathica Systems in the Stress/Septic Response** Kevin Tracy, MD, Cornell University Medical College, Manhasset, New York
11:33 am	**The Role of Adrenomedullin in the Septic Response** Ping Wang, MD, Brown University and Rhode Island Hospital, Providence
10:30 - 11:00 AM **Ballroom Foyer**	**Coffee Available**
12:00-1:00 PM **Ballroom 1 & 2**	**BUSINESS MEETING**
	FREE AFTERNOON

TUESDAY, JUNE 6, 2000

7:00 - 8:00 AM **Ballroom 3, Magpie & Golden Cliff**	**Continental Breakfast**
8:00 - 9:00 AM **Ballroom 3, Magpie & Golden Cliff**	**POSTER SESSION II**, Papers 111- 179
	Cell Signaling, Papers 111-121 Hemorrhagic Shock, Papers 122-152 Neutrophils, Papers 153-161 Nitric Oxide, Papers 162-167 Oxygen Metabolites, Papers 168-169 Pharmacology, Papers 170-174 Ischemia/Reperfusion, Papers, 175-178 Liver, Paper 179
9:00 - 10:00 AM **Ballroom 1 & 2**	**PLENARY SESSION IV**, Papers 180-184 Moderators: **Carol Miller-Graziano, PhD**, University of Massachusetts Medical Center, Worcester and **James A. Thomas, MD**, University of Texas Southwestern Medical Center, Dallas
9:00 am	**Inducible Nitric Oxide Synthase Is Required for Enterocyte Apoptosis After Hemorrhagic Shock**, Paper 180 Evan P. Nadler, MD Children's Hospital of Pittsburgh, Pennsylvania
9:12 am	**IRAK Mediates Postburn Myocardial Contractile Dysfunction**, Paper 181 James A. Thomas, MD University of Texas Southwestern Medical Center, Dallas

Tuesday Continued

9:24 am — **Depressed Trauma Patient MØ IL-18 Levels Lead to Decreased T Cell IL-13 Levels**, Paper 182
Carol Miller-Graziano, PhD
University of Massachusetts Medical School, Worcester

9:36 am — **Inhibition of LPS-Induced ERK ½ Activation and IκBα Degradation by 15-Deoxy-$\Delta^{12,14}$-PGJ_2**, Paper 193
Kelly Guyton, BS
Medical University of South Carolina, Charleston

9:48 am — **Cerebral Perfusion Pressure (CPP) Directed Therapy After Traumatic Brain Injury (TBI)**, Paper 194
Ajai K. Malhotra, MD
University of Tennessee Health Science Center, Memphis

10:00 AM - 12:00 PM
Ballroom 1 & 2

SYMPOSIUM III: Understanding of Myocardial Dysfunction in Hyper Inflammatory States
Moderator: **Kathleen McDonough, PhD**, Louisiana State University, New Orleans

The myocardium is responsible for pumping a cardiac output to match the tissue's requirements for blood flow. Alterations in myocardial function are normally elicited by changes in preload, afterload, contractility and heart rate. However, during inflammatory states, sepsis and compromised myocardial blood flow, changes in myocardial contractile function can occur through other influences such as acidemia, cytokines and chemokines, oxygen radicals and a number of other mediators that my be produced in an inflammatory state. The aim of this symposium is to present an update of t he intracellular mechanisms by which myocardial contractile function is depressed and the role of cytokines in this myocardial depression. In response to injury, the myocardium can upregulate protective functions that serve to blunt the negative consequences of a second insult to the heart. Mechanisms involved in inducing cardioprotection, including the potential role of cytokines, will be discussed. Methods to assess and treat myocardial dysfunction in the clinical setting will be presented. Finally, the issues of potential mechanisms of injury versus mechanisms that have actually been shown to contribute to dysfunction in a pathophysiological state will be discussed.

10:00 am — **Alterations in Myocardial Cell Signaling and Calcium Homeostasis as a Mechanism of Myocardial Depression**
Leona Rubin, PhD, University of Missouri, Columbia

10:24 am — **Cytokine Induced Myocardial Depression and Protection**
Alden Harken, MD, University of Colorado, Denver

10:48 am — **Myocardial Preconditioning by Ischemia and Sepsis**
James Downey, MD, University of South Alabama, Mobile

11:12 am — **Similarities and Difference Between Cell and Whole Heart Models of Myocardial Responses to Sepsis**
Kathleen McDonough, PhD, Louisiana State University, New Orleans

11:36 am — **Advances in Quantifying and Treating Myocardial Dysfunction During Critical Illness: From Bench to Bedside**
Michael Chang, MD, Wake Forest University School of Medicine, Winston-Salem, North Carolina

10:30 - 11:00 AM — **Coffee Available**
Ballroom Foyer

12:00 - 1:30 PM — **Lunch**
Cottonwood 1-4

Tuesday Continued

1:30 - 3:30 PM
Ballroom 1 & 2

WORKSHOP II: Recent Adjuncts to Resuscitation Strategies to Prevent the SIRS to MOF Progression: Bench to Bedside
Moderator: Kenneth Proctor, PhD, University of Tennessee, Memphis

After severe trauma and blood loss, aggressive fluid resuscitation may be the only hope for saving the patient. At the same time, reperfusion promotes reactive oxygen metabolite generation and activates PMNs in splanchnic and other tissues that are already expressing multiple cytokines and endothelial cell surface adhesion molecules. The resultant hyper-inflammatory state can produce secondary injury locally in otherwise undamaged cells, can spill over into remote organs (e.g. lung), or can propagate into a malignant unregulated systemic response leading to SIRS or MOF. One speaker will describe clinically-relevant models of battlefield injuries designed to mimic these conditions. The second speaker will describe the benefits of a novel blood substitute in urban trauma patients, compared to other resuscitation fluids and compared to a potential transfusion-induced cytotoxicity caused by stored, packed RBCs. The third speaker will consider novel strategies in the critically ill trauma patient that combine adequate cellular resuscitation and avoidance of splanchnic vasopressors. Such strategies prevent or ameliorate the ravages of unfettered oxidative stress using agents that attenuate or block unregulated cytotoxin formation and "unprime" PMNs and are initiated in the trauma resuscitation area, ER, or surgical OR. The final speaker will provide an updated review on a number of the clinical trials of new therapeutic agents for the adjuvant treatment of shock, sepsis, and/or SIRS which have just closed to enrollment, are in progress, or are in the final planning stages.

1:30 pm — **Resuscitation Strategies to Minimize End Organ Damage in Large Animal Models of Shock Related MOF**
Kenneth Proctor, PhD

2:00 pm — **Blood Resuscitation: Part of the Solution or Part of the Problem?**
Ernest E. Moore, MD, University of Colorado Health Sciences Center, Denver

2:30 pm — **Resuscitation Strategies to Minimize SIRS & Multiple Organ Failure by Preventing Ischemia-Reperfusion in Trauma Patients**
Orlando Kirton, MD, Hartford Hospital, Hartford, Connecticut

3:00 pm — **Update on Current Clinical Trials of Adjuncts to Resuscitation to Prevent and/or Treat SIRS and MOF**
Mitchell Fink, MD, University of Pennsylvania Medical Center, Pittsburgh

3:00 - 3:30 pm
Ballroom Foyer

Coffee Available

3:30 - 6:00 PM
Ballroom 1 & 2

MINISYMPOSIUM III, Papers 185-196
Moderators: **H. Hank Simms, MD,** Rhode Island Hospital, Providence and **Richard Hotchkiss, MD**, Washington University, St. Louis, Missouri

3:30 pm — **Effects of Fluid Resuscitation in Cerebral Intracellular Calcium in Traumatic Brain Injury Associated with Hemorrhagic Shock**, Paper 185
Marcos Balbino, MD
University of São Paulo Medical School, Brazil

3:42 pm — **Paracrine Regulation of Apoptosis by IL-1β and IL-8-Stimulated PMN: Differential Suppression of FasL and TNF-α Induced Apoptosis**
Paper 186
Patricia S. Grutkoski, PhD
Rhode Island Hospital, Providence

3:54 pm — **TNFR-I is Required for Heat Stress Induction of Cytoprotective HSP70 in MØ**, Paper 187
Julie K. Heimbach
University of Colorado Health Sciences Center, Denver

Tuesday Continued

4:06 pm	**Cerebral Viability After Grade IV Hemorrhage: Is Immediate Fluid Resuscitation Necessary?**, Paper 188 Reza Miraliakbari East Carolina University Brody School of Medicine, Greenville, North Carolina
4:18 pm	**COX-1 Induction and IL1β Expression in Alveolar Macrophages After Unilateral Chest Trauma**, Paper 189 Wesley J. Desselle, MD University of Tennessee Health Science Center, Memphis
4:30 pm	**Alveolar Macrophage TNF-α Release is Enhanced Following Trauma-Hemorrhage and Sepsis**, Paper 190 Doraid Jarrar, MD Rhode Island Hospital, Providence
4:42 pm	**Lethal Septic Shock Increases Myocardial UCP-2 Expression Coincident with Myocardial Dysfunction**, Paper 191 Michael J. Roshon Carolinas Medical Center, Charlotte, North Carolina
4:54 pm	**Mechanisms of PMN Persistence During Inflammation: Suppression of Apoptosis by IL-8 and GRO-α Via Diverse Signaling Mechanisms** Paper 192 Annmarie L. Dunican, MD Rhode Island Hospital, Providence
5:06 pm	**The Dissociation Between Upregulated Endothelins and Hemodynamic Responses During Polymicrobial Sepsis**, Paper 193 David A. Oman, Sc.B Rhode Island Hospital, Providence
5:18 pm	**Immediate Early Genes (IEG) and Transcription Factors in Liver of Rats Preconditioned with Curcumin and Picroliv During Hemorrhagic Shock and Resuscitation**, Paper 194 Gurmel S. Sidhu Uniformed Services University of Health Sciences, Bethesda, Maryland
5:30 pm	**Genetic and Gender Components in the Expression of Tumor Necrosis Factor-α in Mice During Endotoxemia**, Paper 195 F. Dylan Stewart, MD Johns Hopkins School of Medicine, Baltimore, Maryland
5:42 pm	**Two Stage Response to Endotoxin Infusion into Normal Human Subjects**, Paper 196 Fletcher B. Taylor, Jr., MD Oklahoma Medical Research Foundation, Oklahoma City
5:54 pm	**Closing Remarks**

3:30 - 6:00 PM **Ballroom 3**	**MINISYMPOSIUM IV**, Papers 197-208 Moderators: **Allan M. Lefer, PhD**, Thomas Jefferson University, Philadelphia, Pennsylvania and **Lee-Wei Chen, MD**, Veterans General Hospital, Kaohsiung, Taiwan
3:30 pm	**Characterization of Local and Systemic Cytokine Responses During Acute Inflammation in Humans**, Paper 197 Fernando A. Rivera-Chavez University of Texas Southwestern Medical Center, Dallas

Tuesday Continued

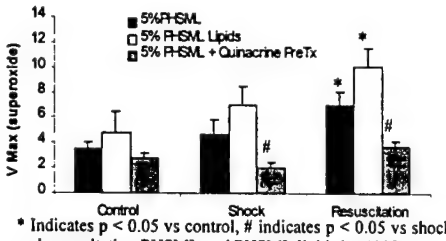
3:42 pm	**Safety and Efficacy of Hypertonic Saline Dextran in Pediatric Patients Submitted to Cardiac Surgery with Cardiopulmonary Bypass**, Paper 198 Roberto Rocha e Silva University of São Paulo, Brazil
3:54 pm	**Prevention of Multiple Organ Failure (MOF) Secondary to Severe Acute Pancreatitis (SAP) with Continuous Hemodiafiltration (CHDF) and Selective Digestive Decontamination (SDD)**, Paper 199 Hiroyuki Hirasawa, MD, PhD Chiba University School of Medicine, Japan
4:06 pm	**Female Gender is a Risk Factor for Early Postinjury Multiple Organ Failure**, Paper 200 Patrick J. Offner, MD Denver Health Medical Center, Colorado
4:18 pm	**Hypoxia Inhibits iNOS Expression in Endothelial Cells**, Paper 201 Haim Bitterman, MD Carmel Medical Center, Haifa, Israel
4:30 pm	**Nitric Oxide Pre-Treatment Protects Against Peroxynitrite-Induced Enterocyte Apoptosis**, Paper 202 Douglas A. Potoka, MD Children's Hospital of Pittsburgh, Pennsylvania
4:42 pm	**The Absence of eNOS Increases Mortality After Hemorrhagic Shock** Paper 203 Vaishali D. Schuchert University of Pittsburgh, Pennsylvania
4:54 pm	**Effects of *n*-Acetylcysteine on Ischemic Brain Injury**, Paper 204 Salvatore Cuzzocrea, PhD University of Messina, Italy
5:06 pm	**Nitric Oxide Synthase Inhibitor Ameliorates Oral Total Parenteral Nutrition-Induced Barrier Dysfunction**, Paper 205 Lee-Wei Chen, MD Veterans General Hospital, Kaohsiung, Taiwan
5:18 pm	**Actin Cytoskeleton and Endothelial Cell Response to Osmotic Stress**, Paper 206 Saman Arbabi, MD Harborview Medical Center, Seattle, Washington
5:30 pm	**Vascular Endothelial Growth Factor (VEGF) Exerts Beneficial Effects in Traumatic Shock Via Preservation of Vascular Endothelial Function** Paper 207 Allan M. Lefer, PhD Thomas Jefferson University, Philadelphia, Pennsylvania
5:42 pm	**Shock Induces Bone Marrow Injury and a Migration of Hematopoietic Precursors to Remote Organs Which is Partially Mediated Through Mesenteric Lymph**, Paper 208 Devashish Anjaria, MD New Jersey Medical School, Newark
5:54 pm	**Closing Remarks**
6:30 - 7:30 PM **Ballroom Foyer**	**RECEPTION**
7:30 - 9:30 PM **Ballroom 1 & 2**	**DINNER**

1

POST-HEMORRHAGIC SHOCK MESENTERIC LYMPH (PHSML) LIPIDS PRIME NEUTROPHIL SUPEROXIDE PRODUCTION VIA PHOSPHOLIPASE A2

Ricardo J Gonzalez*, Ernest E Moore, David J Ciesla*, Christopher C Silliman*, Denver Health Medical Center, Denver, CO 80204

Hemorrhagic shock induced mesenteric hypoperfusion has long been implicated as a key event in the pathogenesis of adult respiratory distress syndrome (ARDS). Previous work links PHSML lipids and enhanced PMN priming in the pathogenesis of acute lung injury. We hypothesize that gut phospholipase A_2 (PLA_2) liberates proinflammatory lipids during hemorrhage and is responsible for enhanced PMN cytotoxicity. **Methods:** Mesenteric lymph was collected from rats (n=5) before(control), during non-lethal hemorrhagic shock (MAP40mmHgx30min), and after resuscitation (shed blood+2xcrystalloid). PMNs were primed with physiologic concentrations (1-5%,v:v) of (a)control, (b)PHSML, (c)PHSML lipid extracts, (d)heat-denatured PSHML and (e)PHSML harvested after IV pretreatment with a known PLA_2 inhibitor (quinacrine,10mg/kg). PMNs were activated by fMLP (1umol), and the maximal rate of superoxide production was measured by reduction of cytochrome C (550nm). **Results:** PHSML and PHSML lipid extracts (5%,v:v) primed for enhanced superoxide production compared to controls. Heat denaturing the PHSML, (eliminating cytokines), had no effect on PMN priming. PHSML collected after PLA_2 inhibition abrogated priming. **Conclusion:** Physiologic concentrations of PHSML lipids prime the fMLP-mediated oxidase. Priming is eliminated with systemic PLA_2 inhibition implicating activation of gut PLA_2 and liberation of proinflammatory lipids as central in the pathogenesis of hemorrhagic shock induced lung injury.



* Indicates $p < 0.05$ vs control, # indicates $p < 0.05$ vs shock and resuscitation PHSML and PHSML lipids by ANOVA

2

NON-COMPARTMENTALIZATION OF GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) FOLLOWING AN INTRAPULMONARY BACTERIAL CHALLENGE.

G.J. Bagby, D.A. Stoltz, P. Zhang, and S. Nelson, LSU Health Sciences Center, New Orleans, LA 70112.

In response to an intrapulmonary infection, pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF) are confined to the bronchoalveolar compartment. We hypothesized that in contrast to TNF, G-CSF produced in the lung following an intrapulmonary *Escherichia coli* challenge would escape the bronchoalveolar compartment since it must reach the bone marrow in order to stimulate granulocyte proliferation in response to infection. To test this hypothesis, 7-wk old BALB/c mice were administered 10^5 live *E. coli* intratracheally and sacrificed 2, 4, 6 or 18 hours later in order to measure TNF and G-CSF in plasma and BAL fluid. As previously observed TNF increased in BAL fluid with highest levels seen at 4 hours after *E. coli* challenge (26 ± 5 ng/ml) but remained low in the plasma (0.08 ± 0.02). In contrast, G-CSF was increased in BAL fluid at 2 hours and increased in BAL fluid and plasma between 4 and 18 hours. At 6 hours after *E. coli*, BAL fluid and plasma G-CSF were 0.54 ± 0.26 and 0.99 ± 0.52 ng/ml, respectively. In a second experiment, *E. coli* was administered intravenously. Under this condition, plasma TNF and G-CSF were substantially increased in the plasma (highest values of 4.86 ± 1.5 and 107 ± 3 ng/ml, respectively) but both cytokines remained less than 0.1 ng/ml in the BAL fluid. These data show that G-CSF, in contrast to TNF, escapes the bronchoalveolar compartment and enters the circulation, which would serve to signal the bone marrow to increase granulocyte proliferation in response to lung infection. The results also indicate that G-CSF movement from the lung into the circulation is unidirectional. (This work is funded by NIH grant AA09803.)

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GLUCOSE-6-P DEHYDROGENASE (G6PD) DEFICIENCY PREDISPOSES TO SEPSIS, WORSENS ANEMIA AND RESULTS IN A PRONOUNCED ACTIVATION OF CIRCULATING MONOCYTES AFTER SEVERE TRAUMA

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G6PD deficiency is the most common human genetic polymorphism. G6PD plays a central role in cellular redox processes. The study tested if G6PD deficient trauma patients have an increased incidence of septic complications and more profound alterations in leukocyte functions compared to non-deficient patients. Male, African American trauma patients were screened for the defect. 44 type A-202/376 G6PD deficient patients were identified and enrolled in the study, together with 43 non-deficient patients with similar age, injury severity and type of trauma. After severe injury, (ISS≅27), 50% of the deficient and 6.2% of non-deficient patients developed sepsis with positive bacterial blood cultures. In deficient patients, the frequency of bronchial (75%) and wound infections (25%) was also increased compared to non-deficient patients (32% and 0%). Whereas ARDS occurred at 30% in both groups, the durations of SIRS, Sepsis Syndrome, days on antibiotics were three times longer in deficient than in non-deficient patients. Anemia was more severe in deficient than non-deficient patients from day10 post-trauma. On day5, peroxide content was doubled, apoptosis was decreased and CD11b expression was increased in monocytes from deficient patients compared to cells from non-deficient patients. On day5, plasma IL-10 levels were significantly lower in G6PD deficient than non-deficient patients. The deficiency was not accompanied by adverse clinical effects after moderate injuries (9<ISS<13). These data indicate that the A- G6PD deficiency predisposes to increased septic complications and worsens anemia in severely injured trauma patients. This adverse clinical course is accompanied by augmented activation status of G6PD deficient monocytes. (Supp. by NIGMS, GM55005).

4

BURN INJURY INDUCES EXPRESSION OF TWO NOVEL FORMS OF THE TIS11D GENE IN MICE. K. Hobson*, K. Cho*, and D. Greenhalgh. Shriners Hospitals for Children Northern California, Sacramento, CA 95817 and University of California Davis, Sacramento CA 95817.

The major cause of death after burn injury is distant organ failure, but the mediators of this systemic effect are poorly understood. Previous work has suggested that early response genes play a crucial role in the pathogenesis of the systemic inflammatory response. To determine which genes participate in this response, reverse transcription polymerase chain reaction (RT-PCR) differential display was used to analyze murine tissue at multiple time points following 18% body surface area burn. TPA inducible sequence 11d (TIS11d), a member of the TIS11 family, was one of the genes demonstrating significant early upregulation. The TIS11 family is a group of genes thought to protect against the systemic inflammatory response by inhibiting normal transcription of TNFα. On subsequent RT-PCR evaluation of mouse lymph node, spleen, thymus, liver and lung tissue after burn, two transcripts of TPA inducible sequence 11d (TIS11d) were found to be upregulated in all burned tissues. In addition, sequence analysis of the upregulated transcripts demonstrated slight changes from the previously described mouse TIS11d gene product. The first transcript contained a 185 base deletion and the second contained a 4 base insertion. Each of these novel transcripts creates a frame shift that, in contrast to the previously described mouse TIS11d sequence, results in an amino acid sequence that bears significant homology to the C-terminus of the human TIS11d protein. These findings suggest TIS11d plays an important early protective role in the systemic response to burn injury in mice. As described in previous studies, it likely exerts these protective effects via inhibition of TNFα transcription. Furthermore, the close homology demonstrated to human TIS11d suggests that the murine model is an appropriate model for use in further studies of the role of TIS11d in human burn injury.

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THE ROLE OF INF-γ AND IL-12 ON PROPIONIBACTERIUM (PA) ACNES-PRIMED LPS HEPATIC INJURY. Y. Shimizu,* N. Otomo,* J.A. Margenthaler, M.D.,* G. Doherty,* and M. Wayne Flye. Wash. Univ., St. Louis, MO.

Administration of the gram-positive bacteria, PA, results in hypersensitivity to subsequent LPS with hepatocyte necrosis. The mechanisms of this injury are still unclear.

Methods: C57BL/6 (B6) or INF-γ deficient (GKO) mice were treated with heat-killed PA (0.5mg/mouse). 7 days later LPS (20μg/mouse) was injected. IL-12 Ab (1 mg/mousex2) was administered to B6 mice either before PA (Group C) or before LPS (Gr. D). Animal survival and plasma levels of INF-γ were followed. Seven days after PA administration and before LPS, liver mononuclear cells ($0.5x10^6$/ml) were cultured for 24 hrs with LPS (10ng/ml) and in vitro cytokine production was measured. Liver mononuclear cells were FACS analyzed by 2-color immunofluorescence (FITC-anti NK1.1 Ab and PE-anti-CD4 Ab). $NK1.1^+$ cells are known to produce INF-γ.

Results: Hepatocyte necrosis, hepatomegaly, and splenomegaly with death developed in Gr. A and D but not in Gr. B and C. INF-γ was correspondingly increased in Gr. A and D.

Gr	Treatment	48 hr survival	INF-γ (pg/ml)*
A	B6 (PA+LPS)	0/12	763 ± 210
B	GKO (PA+LPS)	12/12	0
C	B6 (IL-12Ab +PA+LPS)	8/8	16 ± 11
D	B6 (PA+IL-12 Ab+LPS)	0/8	686 ± 224

*plasma IFN-γ was measured 7 days after PA administration

In vitro cytokine production by liver mononuclear cells

	B6	GKO
IFN-γ (pg/ml)	1064 ± 80	0
TNF-α (pg/ml)	317 ± 96	0
IL-6 (pg/ml)	6580 ± 613	375 ± 127
IL-12 (pg/ml)	70 ± 10	0

Liver mononuclear cell phenotype before and after PA admin.

	B6 (before/after)	**GKO** (before/after)
NK1.1+CD4- cell (%)	13.0 / 38.7	18.1 / 20.8
NK1.1+CD4+ cell (%)	3.5 / 0.9	3.1 / 2.8

Conclusion: Inhibition of IL-12 before the onset of PA-mediated mononuclear cell infiltration reduces subsequent INF-γ production, hepatic injury, and mortality.

6

DEFICIENCY OF DECAY-ACCELERATING FACTOR ATTENUATES LEUKOCYTE-ENDOTHELIUM INTERACTION INDUCED BY HEMORRHAGE AND REINFUSION. R. Scalia, W.C. Song*[1], and A.M. Lefer. Dept. of Physiology, Thomas Jefferson University, Philadelphia, PA 19107 and [1]Center for Experimental Therapeutics, Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Decay-accelerating factor (DAF) is a glycosylphosphatidylinositol-anchored membrane protein that inhibits both pathways of complement activation. In vitro studies have recently shown that DAF functions as a cell adhesion molecule through interaction with the newly identified leukocyte antigen CD97. Accordingly, DAF was also found to be up-regulated in inflammatory conditions. In this study, we investigated the possibility that DAF may play a role in the recruitment of leukocytes during hemorrhage and reperfusion using wild-type and DAF-deficient mice. Mice were hemorrhaged by withdrawal of blood, to a MABP of 40 mmHg for 45 minutes. Mice were then resuscitated by infusion of the shed blood. Leukocyte-endothelium interactions were studied in peri-intestinal venules by means of intravital microscopy. Resuscitation from hemorrhage significantly increased the number of rolling and adherent leukocytes in the splanchnic microcirculation of wild-type mice ($p<0.01$ vs. sham operated control mice). In contrast, mice genetically deficient in the DAF protein, exhibited markedly attenuated leukocyte-endothelium interaction, following hemorrhage and reinfusion. Although the mechanism remains to be defined, these results clearly demonstrate an important role for DAF in the recruitment of leukocytes during acute hemorrhage and reinfusion.

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DOPPLER ECHOCARDIOGRAPHIC ESTIMATION OF CARDIAC OUTPUT IN AWAKE, ANESTHETIZED, AND ENDOTOXEMIC GUINEA PIGS. JR Dodam, LS Hitchcock*, LJ Rubin, and JD Bonagura* Univ Missouri, Columbia, MO 65211.

Doppler echocardiography (DE) is used to non-invasively evaluate ventricular function and can be used to calculate cardiac output (CO). Guinea pigs are used in experimental models of a variety of diseases, but there have been no reports of DE determination of CO in the guinea pig. The purposes of this study were to 1) compare DE estimates of CO to measurements obtained using a transit-time flow meter (TT), 2) determine repeatability of the DE method, and 3) assess lipopolysaccharide (LPS)-induced changes in CO in guinea pigs. CO was measured by DE and TT in eight anesthetized guinea pigs. The TT probe was surgically placed around the ascending aorta, the thorax closed, and DE and TT measurements obtained during four experimental manipulations: baseline, after crystalloid fluid infusion, during infusion of dobutamine, and following bolus administration of pentobarbital. CO was calculated from DE measurements as: CO = aortic velocity-time integral (VTI) x π (aortic diameter $(d_{Ao})/2)^2$ x heart rate. Significant correlation was found between CO measured by DE and TT flow meter (r = 0.76). A higher CO was associated with increased scatter about the regression line. VTI-heart rate product showed higher correlation with TT CO (r = 0.89) suggesting that there was some error in measurement of d_{Ao} using DE. To evaluate repeatability of DE, 10 conscious guinea pigs were used. DE was performed on each guinea pig on 3 consecutive days. Significant differences in CO were not detected among mean daily values for CO. Changes in CO after administration of *E coli* LPS (3 mg/kg, IP) were evaluated in 7 conscious guinea pigs using DE. CO decreased by 21% and 31% at 2 and 4 hours after LPS administration, respectively. This study demonstrates that DE can be used to non-invasively estimate CO in the guinea pig during changing flow conditions.

8

DOES SEXUAL DIMORPHISM EXIST IN SEPSIS?

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Our laboratory has reported that induction of peritoneal sepsis results in a decreased serum concentration of testosterone (T), but unchanged progesterone (P) concentrations in male, Sprague-Dawley rats at 24 hours. We hypothesized that induction of peritoneal sepsis would produce a different steroidogenic response in male and female rats, which might have an influence on overall mortality. Age matched (6-8 weeks) Sprague Dawley Rats were used. Female rats were assigned to four groups coinciding with the stages of the estrous cycle. Proestrous (n=3), estrous (n=2), metaestrous (n=6) and diestrous (n=6) were determined by vaginal smear analysis on the day of experiment. Sepsis was induced with a cecal slurry [200 mg cecal material/kg in 5 ml of 5% dextrose in water (D_5W); ip]. Rats were catheterized and blood samples were collected from the carotid artery before sepsis, 24 hours after sepsis induction, and at 7 days post sepsis induction. Serum concentrations of T, P, corticosterone (C), and estradiol (E) were determined by RIA. Changes in weight and hematocrit levels were similar in all groups. The mortality rate in males was consistent with previous work from our laboratory in this model, and female rats did not differ, except those in estrous (both survived). Sepsis resulted in a significant decrease in serum T concentration in males, and E was decreased in female septic rats (1 and 7 days). The P response pattern was phase dependent, but differed from the pattern seen in males. The C response did not appear to differ between genders. One of the experimental difficulties in obtaining consistent data is the staging of the estrous cycle in the animals. In the present limited study it is concluded that the estrous phase in female rats may be important to overall mortality rate. Sexual dimorphism appears to be cycle dependent, which suggests an importance in overall mortality in sepsis.

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A MURINE MODEL OF INTESTINAL ISCHEMIA-REPERFUSION FOR STUDYING REMOTE ORGAN DYSFUNCTION. Y. Matsuura*, K. Koike, A. Tsujii*, S. Kushimoto* and Y. Yamamoto, Nippon Medical School, Tokyo, Japan

Intesinal ischemia-reperfusion (I/R) has been postulated to play a key role in the pathogenesis of multiple organ dysfunction syndrome (MODS). Animal models to test this hypothesis have been developed in rats but not in mice. There exists more homology between man and mouse with respect to the immune system and various kinds of knockout and transgenic mouse are available. We, therefore, attempted to establish a murine I/R model that is useful for the assessment of remote organ dysfunction. **Method**: Adult C57BL/6 female mice (20g) underwent 45 min of SMA occlusion and were resuscitated with 3 ml of saline injected subcutaneously. After 2h reperfusion whole blood was drawn and organ dysfunction in the intestine, lung and liver was measured by Evans blue (E-B) leak and wet/dry weight ratio (W/D). Liver function was also tested by serum total bilirubin (T-Bil; mg/dL) and ALT (IU/L). The data of E-B method were expressed as the ratio of E-B absorbance to tissue weight (g). (Mean ± SEM. * ; $p<0.05$ by ANOVA, compared with other groups.) **Results**:

		normal	sham	I/R
intest.	E-B	0.70±0.04	0.64±0.03	1.12±0.22*
	W/D	3.88±0.10	3.94±0.09	4.52±0.17*
lung	E-B	1.48±0.04	1.35±0.04	1.98±0.07*
	W/D	3.84±0.15	3.88±0.05	3.91±0.06
liver	E-B	0.55±0.04	0.58±0.07	0.72±0.11
	W/D	2.99±0.02	2.92±0.03	3.16±0.04*
	T-Bil	0.18±0.03	0.17±0.02	0.47±0.07*
	ALT	19±1*	112±28*	205±39*

Conclusion: Each organ dysfunction in the intestine, lung, and liver was quantitated in this murine intestinal I/R model. This model may become a useful tool to delineate the mechanism in the pathophysiology of MODS.

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Organ dysfunction by blunt trauma and a secondary septic challenge- a new two-hit rodent model. H.-C. Pape, M. van Griensven, M. Breddin, F. Böttcher, H. Tscherne Dept. of Trauma Surgery, 30625 Hannover, Germany

Introduction: We developed a reproducible "two hit" small animal model of organ dysfunction by using two different qualities of noxious stimuli. **Methods:** Male NMRI mice (35-45g). In the study group (group 2-Hit), a standardized femur fracture is performed in using a blunt guillotine device (500 g). 24 hours later, cecal ligation and puncture (CLP) is induced (21G needle). Sham: laparotomy without CLP. Animals are sacrificed after 48 or 96 hours. Clinical parameters: Body weight, temperature, food intake, diarrhea (organ failure abdomen. OFA) and piloerection, scoring system for general activity (ACT). Immunologic measurements include FaCs scan of lymphocytic activation (CD4+ and CD8+ cells) and systemic inflammatory reactions of TNF-alpha and Il-1. **Results:**

Group n=10	Body weight	Temp,°C	ACT	CD4/ CD8
Sham	40,44(± 1,15)	38,01 (± 0,42)	1 (± 0)	2.69
2-Hit 48h	35,16 (±1,62)	36,13 ,72)	4 (± 0)	2.30
Sham	42,01 (±8,39)	37,08 (±	1 (± 0)	2.68
2-Hit	32,60 (±7,51)	35,32 (±	3,4(±0,5)	2.0*

The clinical data match with increases of inflammatory parameters (TNFα and IL-10, data not shown). **Conclusions:** In this small animal model, a combination of CLP and a femoral fracture leads to reproducible nonlethal alterations of the clinical status and organ dysfunction, associated with alterations in the specific immune defense systems.

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UP-REGULATION OF TUMOR NECROSIS FACTOR-α IN THE LUNG IN RESPONSE TO THERMAL INJURY IN MICE. L.K. Adamson*, K. Cho*, R.I. Zipkin*, and D.G. Greenhalgh. Shriners Hospitals for Children Northern California, Sacramento, CA 95817.

Complications of sepsis and multiple organ failure remain the leading causes of death among burn patients who survive the initial burn injury. The lungs are often the first target of organ failure as manifested by ARDS. Tumor necrosis factor-α (TNF-α), a proinflammatory cytokine, has been implicated as a prominent inducer of apoptosis and may play a role in the acute inflammation and injury associated with ARDS. To assess whether TNF-α expression after burn plays a role in lung dysfunction, we exposed C57Bl/KsJ mice to an 18% TBSA burn and examined TNF-α and Fas ligand levels in the lung by RT-PCR and immunohistochemistry at various timepoints post thermal injury (control, 3 hours, 1 day, 3 days, 7 days, and 29 days). A two-fold increase of TNF-α mRNA was observed in the lung with a peak expression between 1 and 3 days after thermal injury. Expression returned to basal levels within 29 days post thermal injury. Immunohistochemistry revealed up-regulation of TNF-α in large mononuclear cells (interpreted as macrophages) at timepoints consistent with mRNA data (see below):

Control	Day 1	Day 3	Day 7	Day 29
+	+	+++	+++	+

(+ = minimal expression, +++ = maximal expression)

By contrast, lungs from the same mice expressed Fas ligand constitutively throughout the timepoints. These findings suggest that up-regulation of TNF-α after thermal injury may play an important role in cellular events which lead to lung pathogenesis (e.g. inflammation, apoptosis). Further understanding of the role of TNF-α as well as other molecules in the signaling pathway of lung failure after thermal injury will facilitate the development of medical treatment for burn patients.

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THE SIGNIFICANCE OF ADMISSION HYPOKALEMIA IN TRAUMA PATIENTS. A. Beal * and K. Scheltema * (Spon. G. Beilman) North Memorial Health Care, Minneapolis, MN 55422.

After noting frequent hypokalemia immediately after trauma, we hypothesized that this occurred more frequently in the more severely-injured. A retrospective trauma registry and chart review was done on 552 trauma patients looking at admission potassium, glucose, pH, base excess, specific injuries, and hospital/ICU courses. Admission hypokalemia (<3.6) was more frequent in those who suffered spinal cord injuries (54.5% vs. 33.6%, p=.04) and in those with closed head injuries (40.9% vs. 29.7%, p=.006). Hypokalemia was more frequent in younger patients (28.3 vs. 37.3 yrs., p<.001). The pediatric group, ages 5-14, had admission hypokalemia more frequently than those ages 15-59, or those ages > 59. (52.6% vs. 34.5% vs. 16.7%, p<.001). Admission hypokalemia was more frequent in lower Glasgow Coma Scores (GCS) (12.1 vs. 13.4, p<.001) and higher Injury Severity Scores (ISS) (17.3 vs. 13.6, p=.001). Hypokalemia was a positive predictor of ISS (p=.012). These patients more likely needed a ventilator (p=.006), (26.3% vs. 16.6%, p=.006) but there was only a trend toward more ventilator days (p=.06). Subsequently, hypokalemic patients had longer ICU lengths of stay (2.6 vs. 1.5 days, p=.003) and longer hospital lengths of stay (8.6 vs. 5.5 days, p<.001). Hyperglycemia was more frequent with admission hypokalemia (49.7% vs. 29.9%, p=.001). In summary, initial hypokalemia after trauma is seen more frequently in younger patients, those who have suffered a spinal cord injury or a closed head injury, and in those who are more severely injured, as reflected in a lower GCS and higher ISS. Subsequently, patients with hypokalemia have longer ICU and hospital lengths of stay.

13

AN EXPERIMENTAL MODEL OF BILATERAL TRANSVERSE SINUS PUNCTURE FOR MONITORING HEMISPHERIC CEREBRAL OXYGEN EXTRACTION ($CEREO_2$) DURING BRAIN INJURY AND SHOCK. A. Capone-Neto*, R. Prist*, L.F.Poli Figueiredo, M. Rocha e Silva. – Research Division, Heart Institute- InCor, Univ. São Paulo Medical School, SP 050403-000, Brazil.

Introduction: We developed a bilateral transverse sinus puncture model in dogs for monitoring hemispheric $CerEO_2$ to evaluate the effects of different early fluid resuscitation regimens on systemic and cerebral hemodynamics and oxygen variables. **Methods**: 28 mongrel dogs were submitted to a left parietal cryogenic lesion and controlled hemorrhagic shock to MAP of 40mmHg for 20 minutes and randomized to 5 "pre-hospital" treatment groups: **CT**, controls, **LR 32**, lactate Ringer's 32 ml/kg , **LR 16**, lactate Ringer's 16 ml/kg , **HS 7,5%** , NaCl 7,5% 4 ml/kg and **HS 3%**, NaCl 3% 8ml/kg in 10 minutes. Twenty minutes after starting "pre-hospital" treatment, the "hospital" phase began, when all groups received LR to MAP=70mmHg and shed blood to hemoglobin = 10g%. Cardiac index (CI), intracranial pressure (ICP) and cerebral perfusion pressure (CPP) were monitored as well as arterial, mixed venous, left and right transverse sinus O_2 variables. **Results**: MAP and ICP were significantly higher in the LR groups. There was no significant difference in CPP between groups. During initial resuscitation, $CerEO_2$ followed systemic oxygen extraction in all groups. The injured side showed a trend toward a lower $CerEO_2$ throughout the experiment. Right $CerEO_2$ correlated with CI while left $CerEO_2$ with CPP. **Conclusions**: The experimental model of bilateral transverse sinus puncture allows evaluation of the differences in hemodynamic and O_2 variables between the injured and the non-injured hemisphere.

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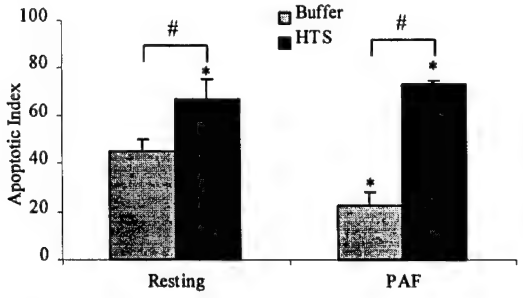
HYPERTONIC SALINE ABROGATES PAF INDUCED DELAYED PMN APOPTOSIS DJ Ciesla,* EE Moore, RJ Gonzalez,* WL Biffl, CC Silliman* Denver Health Medical Center, Denver, CO 80204

Neutrophil (PMN) mediated tissue damage is central to the pathogenesis of postinjury organ dysfunction. Delayed apoptosis contributes to organ injury by impaired clearance of tissue PMNs during postinjury hyperinflammation. Previous work has demonstrated that hypertonic saline (HTS) attenuates PMN cytotoxic functions in vitro and in animal models of hemorrhagic shock. We hypothesized that HTS treatment enhances PMN apoptosis in a proinflammatory environment. **METHODS:** Human PMNs were isolated by dextran sedimentation and density gradient centrifugation. Isotonic (Na^+=140mM) and hypertonic (Na^+=180mM) cell suspensions ($12.5X10^6$ PMN/ml) were incubated at 37°C with and without 10μM PAF. PMN apoptosis was assessed after 24h by ethidium bromide/acridine orange staining of nuclear/cytoplasmic morphology. **RESULTS:** PAF stimulation delayed PMN apoptosis compared to resting controls. HTS treatment increased PMN apoptosis compared to resting controls and abrogated PAF induced delayed apoptosis.(*p<.05 vs resting Buffer, #p<.05vs buffer)



CONCLUSIONS: HTS abrogates imflammatory mediator proviked delayed PMN apoptosis. Improving clearance of tissue PMNs is one mechanism by which HTS resuscitation may attenuate postinjury hyperinflammation.

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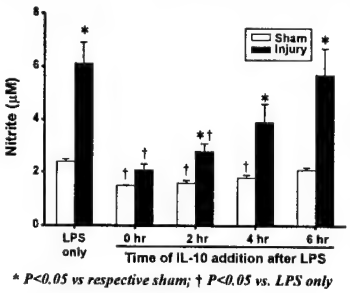
MACROPHAGES ARE PARTIALLY RESISTANT TO THE DOWN-REGULATORY EFFECT OF IL-10 FOLLOWING THERMAL INJURY. M.G. Schwacha, I.H. Chaudry. Center for Surgical Research, Brown University & RI Hospital, Providence RI, 02903.

Thermal injury causes depression of cell mediated immunity and predisposes patients to subsequent sepsis. Recent evidence indicates that activation of a pro-inflammatory cascade is important in for development of the deleterious consequences of thermal injury. Other findings, however, have implicated IL-10, an anti-inflammatory cytokine which can down-regulate macrophage (Mϕ) activity, in post-burn immune dysfunction. The aim of our study, therefore, was to determine the role of IL-10 in the regulation of Mϕ function following thermal injury. Mice were subjected to a third degree burn covering 25% TBSA and splenic Mϕ were isolated 7 days later. LPS-stimulated IL-10, IL-6, and nitric oxide (NO) production were significantly increased in the burn group. When exogenous IL-10 was added to the Mϕ cultures, it dose-dependently suppressed IL-6 and NO production in both groups. The timing of IL-10 addition was, however, crit-ical. If IL-10 was added 2-6 hr after LPS, as opposed to immediately, IL-10's suppressive effect on Mϕ function was attenuated to a greater degree in the thermal injury group as compared to the sham group (P<0.05). These finding suggest that following thermal injury macrophages are partially resistant to down-regulation by IL-10. This desensitization to IL-10 may con-tribute to the thermal injury induced expression of macrophage hyperactivity and subsequent increased susceptibility to sepsis under such conditions. (NIH GM 58242).



** P<0.05 vs respective sham; † P<0.05 vs. LPS only*

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INHIBITION OF TNF-α AMELIORATES BURN-INDUCED LUNG INJURY. J. Schwartz*, C. Schulman*, F. Nwariaku, J. Murphy, B. Giroir, R. Turnage. Univ. of Texas Southwestern Med. Sch. & Dallas VA Med. Ctr., Dallas, TX

Numerous studies have demonstrated that burn injury results in the extravasation of fluid and proteins into the interstitium of the lung. The purpose of this study was to determine the effect of burn injury on pulmonary microvascular permeability and hydrostatic pressure and to determine if inhibition of TNF-α ameliorates burn-induced lung injury. Sprague-Dawley rats were anesthetized and randomized to undergo a 45% TBSA full-thickness burn (BURN; n = 5) or manipulation without injury (SHAM; n = 5). 24-hours later the lungs were excised and perfused *ex vivo* with Krebs-Henseleit buffer. Microvascular permeability was assessed by determining the capillary filtration coefficient (K_f) using a gravimetric technique. Pulmonary vascular resistance (R_t) was determined from the arterial and venous pressure measurements and capillary pressure (P_c) was determined using the double occlusion technique. A second set of animals received the dimeric TNF receptor P80/IgG I Fc fusion protein (sTNFR; 1.75 mg iv; Immunex) prior to burn injury; 24-hours later burn-induced changes in K_f and R_t, and P_c were determined. Data are expressed as mean ± SEM and analyzed by ANOVA.

	SHAM	BURN	BURN + sTNFR
K_f	0.005 ± 0.0005	0.012 ± 0.001*	0.006 ± 0.0006
R_t	1.08 ± 0.03	1.00 ± 0.044	0.812 ± 0.0157
P_c	5.6 ± 0.2	5.25 ± 0.08	5.550 ± 0.4010

Kf expressed as gm/min/mmHg/100 gm wt; Rt expressed as mmHg/ml/min/100 gm wt *p < 0.05 vs either SHAM or BURN + sTNFR

Burn injury induced a greater than 2-fold increase in K_f when compared with uninjured controls. In contrast, there was no change in R_t or P_c. This change in microvascular permeability could be totally prevented by pre-treating the animals with the TNF-α inhibitor, sTNFR suggesting that TNF-α is an important mediator of the microvascular changes associated with burn-induced lung injury.

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POSTBURN SERUM ACTIVATES L-TYPE CALCIUM CHANNEL IN ISOLATED CARDIAC MYOCYTES OF ADULT RATS. H. Shen*, Y. Chen*, J.W. Horton, Z. Xia. Shanghai Hosp., Changhai, China and UTSWMC, Dallas, TX 75390-9160.

INTRODUCTION: The present study was designed to evaluate the effect of postburn serum (BS) on L-type calcium channels in isolated cardiac myocytes; we hypothesized that burn trauma alters cardiomyocyte function by targeting these channels, contributing to postburn intracellular calcium overload. **METHODS:** Cardiac myocytes were harvested from adult Sprague-Dawley rats, and challenged with BS collected (at several times) from rats with 30%TBSA full thickness burn trauma and fluid resuscitation. L-type calcium currents ($I_{ca\text{-}L}$) were recorded in a whole-cell patch-clamp model. **RESULTS:** With BS stimulation of cardiomyocytes (BS collected 2 hrs or 6 hrs postburn), the peak $I_{ca\text{-}L}$ increased 50 and 80% (1.5 and 2.5 folds increases) respectively (Fig.1). The peak current-voltage curve (Fig. 2) showed increases of I_{ca} under the voltages controlled by the depolarizing procedure, and left shifted maximum triggering voltage. All changes induced by application of burn serum were abolished by washing the cells with extracellular solution. **CONCLUSION:** Burn serum increases the calcium inflow by activating L-type calcium channels, providing one mechanism by which burn trauma promotes cardiomyocyte calcium accumulation.

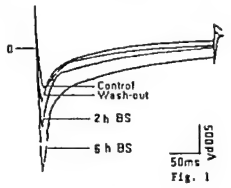


Fig. 1

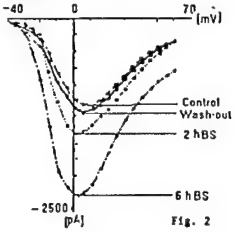
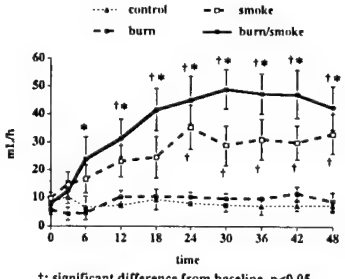


Fig. 2

18

PATHOPHYSIOLOGICAL ANALYSIS OF COMBINED BURN AND SMOKE INHALATION INJURY IN SHEEP. L. Traber, K.Soejima*, J. Katahira* and D. Traber, Univ. Texas and Shriners Burns Institute, Galveston, TX 77555-0833

We investigated pathophysiological alterations seen with combined burn and smoke inhalation injuries by focusing on pulmonary vascular permeability compared with either burn alone, smoke inhalation injury alone or no injury with same experimental setting. METHODS: Sheep (n=24) were prepared for chronic study with lung lymph fistula. The animals were divided to a burn group (received 40% third degree burn alone, n=6), a smoke group (received 48 breath of smoke from burning cotton, n=6), a burn/smoke group (received both burn and smoke, n=6) and a control group (no injury, n=6).



RESULTS: Lung lymph flow was significantly higher in the burn/smoke group than in the burn alone group. The lung edema formation was most severe in the combined injury group (Wet/Dry ratios of the lung were burn/smoke > smoke > burn > control). However, pulmonary microvascular permeability to protein was similar in both the smoke and burn/smoke group. CONCLUSION: The results suggest that burn injury dose not contribute to protein leakage from pulmonary microvasculature, even when the burn is associated with smoke inhalation injury. The lung edema formation that is more severe than either burn alone or smoke inhalation injury alone is probably due to changes in permeability to water and small molecules.

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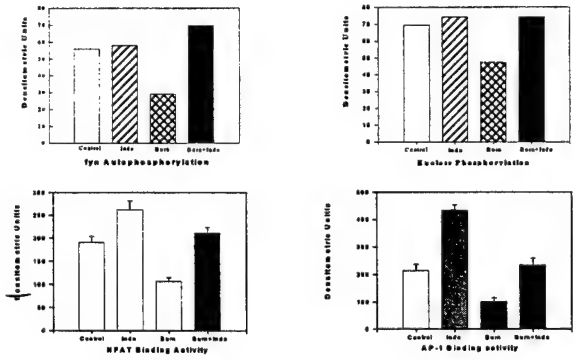
EFFECTS OF PROSTAGLANDIN E2 ON SPLENIC T CELL SINGALING DURING BURN INFLAMMATION

H.Mao*, X.Ren*, M.A.Choudhry, and M.M.Sayeed, Burn & Shock Trauma Inst., Depts.of Surgery and Physiology, Loyola University Chicago Medical Center, Mayword, IL 60153

Prostaglandin $E_2(PGE_2)$ is known to suppress immune functions including T cell activation and IL_2 production in inflammatory conditions. We have evaluated whether PGE_2-mediated suppression in T cell proliferation during burn injury could result from attenuations in $P59^{fyn}$ kinase activity and IL_2 specific nuclear factors, NFAT/AP-1. Splenic T cells were isolated from control and burn(3rd degree, 25% TBSA) rats. T cells were stimulated with ConA/anti-CD_3. Fyn autophosphorylation and kinase activation were measured by immunoprecipitation and in vitro kinase assay, and DNA binding activity of NFAT/AP-1 were measured using electrophoretic mobility shift assays.



The data show decreases in autophosphorylation and kinase activity of fyn accompanied by decreases in NFAT/AP-1 binding activities in T cells from burn animals. The burn-related inhibition of $P59^{fyn}$ and NFAT/AP-1 activities could be due to endogenous PGE_2, as PGE_2 inhibition via indomethacin seemed to prevent such decreases. (supported by NIH grants GM 53235 and GM 56865)

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BETA-1-ADRENOCEPTOR-AGONISTS SPECIFICALLY UPREGULATE THE STRESSPROTEIN HEME OXYGENASE-1 IN PRIMARY CULTURES OF RAT HEPATOCYTES.

H.Rensing*, I. Bauer*, M. Paxian*, M.Bauer Dept of Anesthesiology, University of the Saarland, D-66421 Homburg, FRG.

We have previously shown that hepatocellular induction of the stress protein heme oxygenase(HO)-1 maintains liver blood flow and attenuates liver injury after hemorrhagic shock in vivo (1). Previous reports suggest that increased intracellular cAMP levels induce HO-1 via a protein kinase A (PKA) dependent pathway (2). Adrenoceptor-agonists may increase intracellular cAMP levels. Aim of the present study was to assess the role of adrenoceptor-agonists for induction of HO-1 in primary cultures of hepatocytes. Hepatocytes were isolated from Sprague-Dawley rats by collagenase perfusion and density centrifugation. Cells were cultured under air/CO_2 (19:1) in Williams E Medium. Fetal calf serum (5%) was present during the plating phase up to 4 hours, and cell cultures were incubated in serum free medium for 18 h before treatment. Induction of HO-1 was assessed by standard Northern blot. The β1-adrenoceptor-agonist dobutamine (7.5, 75 and 750μM) induced HO-1 in a time and dose dependent manner. No induction of HO-1 was observed after α1-, α2- or β2-agonists. Pretreatment with the β1-antagonist metoprolol (100μM) attenuated HO-1 induction by dobutamine. The PKA inhibitor H89 (50μM) abolished induction by dobutamine and 8Br-cAMP (1mM). These results suggest a specific induction of HO-1 by β1-agonists via the PKA signaling pathway in hepatocytes. The ability of β1-agonists to stimulate the HO-catalyzed production of the vasodilator carbon monoxide may play an important role in liver blood flow regulation.

(supported by DFG grant (Ba1601/1-2). (1) Rensing et al. Crit Care Med 27:2766-75 (1999); (2) Immenschuh et al. Molecular Pharmacology, 53: 483-91 (1998).

21

ROLE OF BCL-2 IN PEROXYNITRITE-MEDIATED CELL DEATH: POLY (ADP-RIBOSE) SYNTHASE DEPENDENT AND INDEPENDENT PATWHAYS

L. Virág and C. Szabó, Inotek Corporation, Beverly, Massachusetts, Department of Medical Chemistry, University Medical School of Debrecen, Hungary, Division of Critical Care, Children's Hospital Medical Center, Cincinnati, Ohio

In thymocytes, peroxynitrite induces poly(ADP-ribose) synthetase (PARS) activation which results in necrotic cell death. In the absence of PARS, however, peroxynitrite-treated thymocytes dye by apoptosis. As Bcl-2 has been reported to inhibit not only apoptotic but also some forms of necrotic cell death, here we have investigated how Bcl-2 regulates the peroxynitrite-induced apoptotic and necrotic cell death. We have found that Bcl-2 did not provide protection against peroxynitrite-induced necrotic death, as characterized by propidium iodide uptake, mitochondrial membrane potential (MMP) decrease, secondary superoxide production and cardiolipin loss. In the presence of a PARS inhibitor, peroxynitrite-treated thymocytes from Bcl-2 transgenic mice showed no caspase activation, DNA fragmentation and displayed smaller MMP decrease. These data show that Bcl-2 protects thymocytes from peroxynitrite-induced apoptosis at a step proximal to mitochondrial alterations, but fails to prevent PARS-mediated necrotic cell death. Activation of tissue transglutaminase (tTG) occurs in various forms of apoptosis. Peroxynitrite did not induce transglutaminase activity in thymocytes and did not have a direct inhibitory effect on the purified tTG. Basal tissue transglutaminase was not different in Bcl-2 transgenic and wild type cells.

22

PRETREATMENT WITH CURCUMIN MODIFIES CYTOKINE EXPRESSION DURING HEMORRHAGIC SHOCK AND RESUSCITATION IN RATS. Jaya P. Gaddipati*, Jillian Calemine*, Haresh Mani*, Pankaj Seth*, Gurmel S. Sidhu* and Radha K. Maheshwari*. (Spon: Florence M Rollwagen). Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Proinflammatory cytokine cascades are initiated following hemorrhagic shock and are widely implicated in organ dysfunction. Hemorrhage-induced increases in tissue cytokine contents are organ specific and differential elevations have been noted in heart, spleen, lung, liver, kidney, gut and brain. Significant hemorrhage carries considerable death rate regardless of intervention. Curcumin (diferuloymethane) has been shown to have pleiotropic biologic activities including inhibition of neutrophil activation, suppression of mononuclear cell proliferation. We have tested curcumin for the prevention of hemorrhagic shock injury and preliminary data indicate a significant survival advantage by pretreatment with curcumin. In the present study, we have compared the cytokine gene expression in various organs during hemorrhagic shock and resuscitation and the response to curcumin pretreatment. Shock was initiated in anesthetized rats by bleeding of 30 ml per kg body weight from the femoral artery. After one hour, the rats were resuscitated with 2X volume of lactated Ringer's solution. The animals were sacrificed 2 h of post-resuscitation and liver, intestine, kidney, lung, brain, heart and spleen were harvested. Total RNA was extracted and cytokine mRNA (IL-1α, IL-1β, IL-2, IL-6, IL-10 and TNF- α) was analyzed by semi-quantitative RT-PCR. The results demonstrate that the cytokine profiles may be quite different between organs. The data also suggest that pretreatment with curcumin is effective in inhibiting some of the proinflammatory cytokines. (Supported by Office of Naval Research Grant G174HV).

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COMPARISON OF CYTOKINE mRNA EXPRESSION IN DIFFERENT ORGANS IN A RAT TWO-HIT MODEL. M.Grotz*,H.C.Pape,M.v.Griensven*,H.Tscherne* (Spon: E.A. Deitch). Unfallchirurgie, MHH, 30623 Hannover, Germany

The gut liberates cytokines after intestinal I/R as well as ET-challenge. The aim of this study was to compare the cytokine mRNA expression of the gut with different other organs (lung/liver) in a two hit MOF model. Methods: Rats were subjected to occlusion of the sup. mesenteric artery for 45 min (SMAO) and i.p. ET/NaCl challenge 6 hrs later. The control group (CON) consisted of uninstrumentated rats. Expression of mRNA of TNF, IL-1ß, IL-10 (ag/fg GAPDH mRNA) in lung, liver, ileum were determined by competitive RT-PCR at 1 hour after ET/NaCl challege. 24 hrs mortality rate was recorded. Statistics: Data are presented as means±SEM, Chi-square-test, Student-t-test; *p<0.05 vs. other organs; +p<0.05 vs. SMAO/NaCl; § below limit of detection. Results: 24 hrs mortality: SMAO/ET: 5/12; SMAO/NaCl: 0/12; CON: 0/6

		lungs	liver	ileum
TNF mRNA:	SMAO/ET	18.7±5.3*	5.3±3.4+	12.4±6.1+
	SMAO/NaCl	12.5±9.9*	0.2±0.1	1.1±1.0
IL-1 mRNA:	SMAO/ET	29.5±15.9*+	7.4±4.2+	2.1±0.4
	SMAO/NaCl	0.1±0.1	0.2 ±0.1	§
IL-10 mRNA:	SMAO/ET	49.8±9.1*+	28.1±1.5+	1.4±0.6
	SMAO/NaCl	§	§	§

All cytokine mRNA values of the CON group were below the limit of detection. Conclusions: The lung shows for all cytokines highest mRNA expression after intestinal I/R and ET-challenge, solely for TNF the ileum shows comparable values. The cytokine mRNA expression is significantly increased after SMAO/ET in comparison to the SMAO/NaCl group. In summary this study confirms the concept, that intestinal mediators reach the systemic circulation via the intestinal lymphatic duct and not the portal vein and therefore lead to a damage of the lung, rather than the liver.

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CONTROL VALUES OF PRO- AND ANTI-INFLAMMATORY MEDIATORS IN PLASMA AND CEREBROSPINAL FLUID

Herzog C, Maier B*, Larsen R*, Marzi I. ;Depts. of Trauma Surgery and Anesthesiology, Medical School, 66421 Homburg/Saar, Germany.

Increased pro- and anti-inflammatory activity in plasma [P] and cerebrospinal fluid [CSF] is frequently seen following traumatic brain injury, sepsis [e.g. bacterial and viral meningitis], chronic infection [e.g. HIV], multiple sclerosis and neurosurgical procedures. The aim of our study was to establish control values of healthy humans for the pro-inflammatory mediators Interleukin [IL]-6, -8, sICAM and the anti-inflammatory mediators IL-10, soluble [s] TNF-Receptor [55/75kD] and sE-Selectin in CSF. Material and Methods: After receiving informed consent, paired samples of CSF [native] and P [EDTA] were taken from patients undergoing elective surgery in spinal anaesthesia. IL-6, -8, and -10 were measured using commercial ELISA kits; sTNF-Receptors [55/75kD], sICAM and sE-Selectin were analysed using ELISA kits according to Laan MP et al.; Allergy, 1998; 53 (1), 51-8. Results: Samples from 113 patients, 81 male, 32 female, 22 to 89 years [58,4+17,7], were measured. Significant differences between sexes and age were not found. Data are given as median and 5-95% confidence interval. IL-6: P: 5; 1-29 pg/ml; CSF: 7; 1-23 pg/ml. IL-8: P: 8; 5-18 pg/ml; CSF: 42; 15-77 pg/ml. IL-10: P: 11; 4-27 pg/ml; CSF: 1; 0-2 pg/ml. sTNF-R 55kD: P: 2; 1-4 ng/ml; CSF: 1; 0-3 ng/ml. sTNF-R 75kD: P: 1; 0-2 ng/ml; CSF: 0,3; 0-1 ng/ml. sICAM: P: 91; 55-113 ng/ml; CSF: n.d. sE-Selectin: P: 11; 4-19 ng/ml; CSF: n.d. Conclusion: Values of IL-6 and IL-8 in CSF are significantly higher (p<0,001) than corresponding plasma values whereas values of IL-10 and sTNF-R [55/75 kD] are significantly lower (p<0,001). sICAM and sE-Selectin, detectable in P, were not found in CSF. This imbalance of pro- and anti-inflammatory mediators in healthy humans coincidences with our findings of highly elevated cytokine levels, e.g. after traumatic brain injury IL-6 increases up to 5017±440pg/ml in CSF in contrast to IL-10 with levels of 54±28 pg/ml [Marzi I et al.: Hefte zu Unfallchirurg 99, 11.99].

25

DOPAMINE DOES NOT RESTORE VO_2/DO_2 ABNORMALITY DURING VASOMOTOR SHOCK INDUCED BY IL-1β. Y. Kuwagata, J. Oda*, S. Matsuyama*, M. Nishino and H. Sugimoto*. Osaka Univ. Med. Sch., Osaka 565-0871, Japan.

We have shown that IL-β induces a vasomotor shock and impairs VO_2/DO_2 relationship by increasing the slope of the supply-independent line in rabbits. In the present study, we investigated the inotropic effect of dopamine on the VO_2/DO_2 abnormality induced by IL-1β. Twelve rabbits were randomly divided into two groups (n = 6, each) and given 10 μg/kg of IL-1β or saline (Ctrl) intravenously. After baseline measurements, dopamine was continuously infused at a rate of 20 μg/kg/min throughout the study in both groups. All rabbits were subjected to stepwise cardiac tamponade to reduce DO_2 down to 5 ml/kg/min by inflating a handmade balloon placed into the pericardial sac. The VO_2/DO_2 relationship was analyzed by the dual line method. Dopamine significantly increased DO_2 in both groups (IL1β: 28.9 ± 5.7 ml/kg/min from baseline 25.3 ± 3.8 ml/kg/min, Ctrl: 26.6 ± 2.0 ml/kg/min from baseline 21.6 ± 2.2 ml/kg/min), but did not affect VO_2 (IL1β: 10.3 ± 1.9 ml/kg/min from baseline 10.3 ± 1.9 ml/kg/min, Ctrl: 9.6 ± 0.8 ml/kg/min from baseline 9.5 ± 1.1 ml/kg/min). The IL-1β group showed significantly less mean arterial pressure (73 ±8 mmHg vs 83 ± 7 mmHg) and significantly greater cardiac index (191 ± 24 ml/kg/min vs 125 ± 16 ml/kg/min) than Ctrl at the first experimental measurement without cardiac tamponade. The IL1β group showed significant greater slopes of the supply-independent line than Ctrl (IL1β: 0.15 ± 0.05, , Ctrl: 0.08 ± 0.02) during the stepwise decrease in DO_2. These results indicate that the continuous infusion of dopamine at 20μg/kg/min increased DO_2 up to supranormal level, but failed either to improve the vasomotor disturbance or to restore the VO_2/DO_2 abnormality induced by IL-1β.

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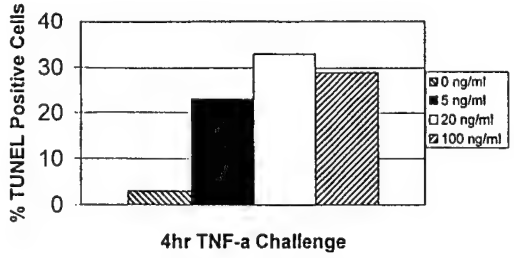
TNF-α INDUCED APOPTOSIS OF CARDIOMYOCYTES. E. Lightfoot Jr.*, J. W. Horton, D. Maass*, B. Giroir. U.T. Southwestern Med. Ctr., Dallas, TX 75390-9160.

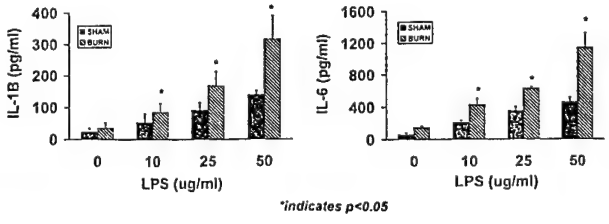
Our previous studies have confirmed that major burn trauma produces coronary endothelial injury, apoptosis of cardiomyocytes, and cardiac contractile defects. Additional studies from our lab have shown that burn trauma promotes TNF-α secretion by cardiomyocytes, producing local levels of inflammatory cytokine that exceed those in the systemic circulation. In this study, we examined the concentration and time related incidence of apoptosis caused by TNF-α in cardiac myocytes. Ventricular myocytes were isolated from normal adult rats (collegenase digestion), suspended in Tyrodes buffer and exposed to several concentrations of rat recombinant TNF-α for 4 or 18 hrs. Cells were incubated in a CO_2 incubator, and apoptosis was quantitated by fluorescent TUNEL and ANNEXIN V staining. As shown in the Figure, there was a concentration dependent apoptotic response to TNF-α in cardiomyocytes. Exposure of the cardiomyocytes to 20 ng/ml of TNF-α increased the number of apoptotic cells from 3% (seen in the absence of TNF-α) to 33%. From our data, we conclude that injury states such as burn trauma may promote cardiomyocyte secretion of TNF-α which, in turn, may produce apoptosis of cardiomyocytes, contributing to the cardiac contractile dysfunction that has been shown to occur after burn injury.



27

BURN TRAUMA STIMULATES CARDIOMYOCYTE SECRETION OF INTERLEUKINS (IL-1β AND IL-6). D. Maass* and J. W. Horton. UTSWMC, Dallas, TX 75390.

Burn trauma increases secretion of TNF-α by cardiomyocytes, and anti-TNF strategies given after burn decreases cardiac TNF synthesis and improves cardiac function (Giroir, Horton et al., AJP 267:H118-124, 1994). Work by others has suggested that TNF-α is an early inflammatory mediator which upregulates synthesis of additional cytokines such as IL-1β. Thus, the interleukins may serve as final mediators of cardiac dysfunction after major trauma. This study examined the effects of burn trauma on cardiomyocyte secretion of IL-1β and IL-6. Sprague Dawley rats were given a 3° scald burn over 42% TBSA (sham burns for controls) and fluid resuscitated with lactated Ringer's, 4 ml/kg/% burn. Rats were sacrificed 24 hrs postburn, hearts were perfused with collegenase containing buffer. Isolated myocytes from sham and burned rats (5 x 10^4 cell/ml), were pipetted into microtiter plates, and stimulated with LPS (0, 25 and 50 mg, LOT# 65H 4053, DIFCO Laboratories) for 18 hrs (CO_2 incubator at 37°C). IL-1β and IL-6 were measured in supernatants (ELISA). Burn trauma increased synthesis of IL-1β and IL-6 by cardiomyocytes in the absence of LPS; LPS challenge exacerbated burn-induced cardiac interleukin synthesis by cardiomyocytes ($p<0.05$). Our data confirm that burn trauma upregulates interleukin secretion by cardiomyocytes; the specific contribution of IL-1β and IL-6 to postburn cardiac dysfunction warrants further study. Supported by NIH Grant (GM 21 681-35).



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PROLONGED BLOCKADE OF BOTH TNF AND IL-1 IMPROVES SURVIVAL FOLLOWING CECAL LIGATION AND PUNCTURE. D. Remick, D. Call, S. Ebong, D. Newcomb, P. Nybom, J. Nemzek, and G. Bolgos
Dept Pathology U of Michigan Ann Arbor Michigan 48109

Blocking TNF or IL-1 alone has not improved sepsis survival in human clinical trials. Therefore it has been suggested that blockade of both may be successful. Mice were treated with the combination of the IL-1 receptor antagonist (IL-1ra) plus a polyethylene glycol-linked dimer of the TNF soluble receptor (TNF-SR) by subcutaneous (subq) injection followed by an intraperitoneal challenge with a lethal dose of lipopolysaccharide (LPS). The short half-life of the IL-1ra necessitated additional treatment at 3, 7, and 11 hours. Treatment resulted in reduced mortality and a decrease in biologically active plasma and peritoneal TNF. A similar treatment regime was tested in the cecal ligation and puncture (CLP) model of sepsis. CLP was performed with a 21 gauge needle and all mice were treated with fluids and antibiotics. Blockade of both TNF and IL-1 decreased plasma and peritoneal levels of IL-6 and the murine chemokines MIP-2 and KC 8 hours after CLP, a time of near peak cytokine levels. However, treatment did not result in a reduction in the hypothermia or peripheral blood alterations which occur following CLP. When mice were treated for the first 11 hours there was no improvement in overall survival but there was a delay in lethality. Therefore, mice were treated every day for 3 days with TNF-SR by subq injection and IL-1ra was administered continuously subq via osmotic pump for 3 days. With prolonged combination immunotherapy there was a significant improvement in survival (alive/total vehicle = 11/32, treatment 17/32, p=0.04 differences between the groups by log rank survival analysis). These data demonstrate that the compounds are capable of decreasing the lethality of sepsis initiated by CLP if combination treatment is provided for a sufficient length of time.

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SERUM INTERLEUKIN-18 CONCENTRATIONS IN PATIENTS WITH MULTIPLE ORGAN DYSFUNCTION SYNDROME. N. Sato*, K. Koike, T. Masuno*, T. Mochizuki*, S. Kushimoto*, Y. Koido*, M. Kawai*, Y. Yamamoto, Nippon Medical School, Tokyo, Japan

Interleukin-18 (IL-18) is a recently cloned cytokine that plays important roles in inflammatory reactions. We measured serum IL-18 levels serially in patients with multiple organ dysfunction syndrome (MODS) comparing to various clinical parameters. **Method:** IL-18 levels (pg/ml) were determined by ELISA in 24 patients with MODS (male 19, female 5; age 17-92; survivors 10). The highest IL-18 concentrations during the hospital stay were compared with APACHE II score, sequential organ failure assessment (SOFA), lung injury score (LIS), PaO_2/FiO_2, T.Bil, ALT, WBC, CRP on the same day. (mean±SEM, Mann-Whitney U-test and Spearman's rank correlation,*p<0.05.) **Results:** The highest IL-18 concentrations: MODS vs. normal; 646±317 vs. 126± 45*; MODS with persistent infection (PI;> days) vs. MODS with non-PI; 924 ± 286 vs. 481 ± 199*. survivors vs. non-survivors; 103±10 vs. 197±14. IL-18 vs. LIS (r=0.547*). PaO2/FIO2 (r=0.475*), APACHE II (r=0.002), SOFA (r=0.207), T.Bil (r=0.117), ALT (r=0.009), WBC (r=0.222), CRP (r=0.212). **Conclusion:** Serum IL-18 concentrations appear to relate with respiratory function and were high in patients suffered from persistent infection.

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GUT-DERIVED NOREPINEPHRINE (NE) UPREGULATES TNF-α PRODUCTION.
M. Zhou, D.J. Koo*, D.A. Ornan*, I.H. Chaudry and P. Wang. Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Previous studies have indicated that systemic levels of TNF-α increase during the early stage of sepsis. However, the mechanism responsible for upregulation of this cytokine remains unknown. Since the recent studies have shown that the gut is an important source of NE release during early sepsis, we attempted to determine whether gut-derived NE plays any role in upregulating TNF-α. To study this, a branch of the portal vein was cannulated with PE-10 tubing in normal adult male rats under anesthesia. NE (20 μM in 0.1% ascorbate saline solution), NE with Yohimbine (YHB, an α_2-adrenoceptor antagonist, 1 mM) or 0.1% ascorbate in saline (vehicle) were infused at a rate of 13 μl/min for 2 h. This procedure did not cause any apparent gut ischemia. Following the infusion, blood samples were collected by cardiac puncture. Kupffer cells (KC) were harvested by collagenase perfusion and purification. Plasma and KC levels of TNF-α were measured by ELISA. TNF-α gene expression in KC was examined by RT-PCR. The serum and KC levels of TNF-α (pg/ml serum or pg/10^6 cells; n=5-6/group; mean ±SE) are as follows:

	Serum	Kupffer Cells
Vehicle	21.5±15.9	16.8±2.9
NE	286.9±110.3*	25.9±1.9*
NE+YHB	46.1±28.5#	14.1±0.7#

(ANOVA & Tukey's: *P<.05 vs Vehicle; #P<.05 vs NE) The results indicate that serum and KC levels of TNF-α were significantly increased after portal infusion of NE at a concentration similar to that observed during sepsis. Co-administration of YHB and NE, however, attenuated TNF-α production. In addition, KC TNF-α gene expression was increased in NE-infused animals. Thus, gut-derived NE upregulates TNF-α production in KC through an α_2-adrenoceptor pathway, which appears to be responsible for the elevation of circulating TNF-α during early sepsis.

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ROLE OF SIALIC ACID ON RED BLOOD CELL SHAPE IN SEPSIS. Piagnerelli M[1], Zouaoui Boudjeltia K[2], Vanhaeverbeek M[2], Vincent JL[3], Carlier E[1], Lejeune P[1].
[1]Intensive Care Unit, [2]Experimental Medicine Laboratory, CHU A.Vésale, Montigny-le-Tilleul and [3] Dpt of Intensive Care, Erasme University Hospital, University of Brussels. Belgium.

Background : Changes in red blood cell (RBC) shape and increase in RBC aggregability are two common events in sepsis. In diabetic

patients, a decrease in RBC deformability has been related to a decrease in the negative charge on the RBC membrane, secondary to a reduction in sialic acid (SA) content (1). We hypothesized that similar changes may occur in sepsis.
Objective : To study the relationship between RBC shape and SA content of the RBC membrane in septic patients.
Methods : We studied blood samples in control volunteers (n=14), and in ICU patients without (n=13) and with (n=12) documented sepsis. SA was measured on isolated RBC membrane protein, by an enzymatic colorimetric assay (sialic acid; Boehringer®), adapted for microdeterminations. To evaluate RBC shape, we calculated a spherical index using light scattering in flow cytometry (a perfect spherical shape would have an index of 1).
Results : The SA content was lower in the septic patients (p=0.036) than in the other groups, and the changes in RBC shape were significantly correlated with the SA content (R= 0.69, p = 0.01 , Spearman test)
Conclusions : Decreased SA content in the RBC membrane may be implicated in the alterations in RBC shape in septic patients, and contribute to RBC rheologic alterations in sepsis.
Reference: (1) Clinical Science (1992) 82, 309-313.

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MYOCARDIAL DEPRESSION AFTER ENDOTOXIN IN AWAKE SHEEP. F. L. Abel, P. Kroesl, H. Redl, K. Kropik,* and G. Schlag. Univ. So.Carolina Sch. Med., Columbia, SC 29208 and Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria.

Endotoxin is responsible for much of the pathology of sepsis and traumatic shock. These experiments were undertaken to document its role in cardiovascular depression in a minimal dose in unanesthetized animals. Eight previously instrumented Austrian mountain sheep (ave. wt. 38 kg) received a dose of 20 ug/kg/min for 10 hours. Aortic pressure, pulmonary artery pressure, left ventricular pressure and volume, and thermodilution cardiac output were recorded at 30 min intervals for 10 hours and again at 24 hours. Blood was drawn for counting, blood gases, and TNF-alpha. Analog pressure and volume recordings were used to calculate the parameters of ventricular function.

Results: There was an early increase in TNF-alpha and pulmonary arterial pressure. However, except for a small increase in heart rate at 8 hours, there were no significant changes in arterial pressure, cardiac output, cardiac work, systolic or diastolic volumes, or end diastolic pressure. Despite these normal findings, careful analysis of the data indicated cardiac contractile depression by maximal dp/dt (at a constant afterload, preload, and heart rate) at four hours and lasting until after 10 hours. Variables such as end systolic elastance and Pmax/EDV also showed depression at 6 and 9 hours. Relaxation time was decreased by 6 hours.

We conclude that at very low levels of endotoxin, despite lack of the usual changes in cardiac variables, there is evidence for early depression of the left ventricle, which might easily escape detection.

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Effects of fluid resuscitation on the pattern of vascular gene expression during abdominal sepsis. R Baveja*, N Kresge*, Y Yokoyama*, N Sonin*, JX. Zhang*, T Huynh# and MG Clemens*. *Biology, Univ North Carolina Charlotte, and #Surgery, Carolinas Med Center, Charlotte NC.

Hepatic failure is associated with changes in the vascular gene expression and microcirculatory dysfunction in sepsis. Controversy exists regarding the amount of fluid resuscitation needed to limit the organ damage due to sepsis. It is unknown if the vascular gene expression is dependent on the volume of resuscitation. To study this, rats (350-450g) were subjected to sepsis by cecal ligation and puncture (CLP). These and sham rats received either 2.5 or 5 ml/100g BW normal saline SQ. At 24 hour after CLP, the systemic hemodynamic was assessed before sample retrieval. RT-PCR was performed to measure the vasoconstrictor genes endothelin-1 (ET-1), its receptors ET_A and ET_B and vasodilator genes inducible and constitutive nitric oxide synthase (iNOS and eNOS respectively) and hemeoxygenase-1 (HO-1). The hematocrit was increased in CLP with a higher increase in low volume resuscitation (LVR) as compared to high volume resuscitation group (HVR) (56±1.9 in HVR; 61.6±0.5 in LVR). ET-1 and eNOS mRNA increased in CLP with a higher response in HVR as compared to LVR groups (4.2 and 1.2 fold increase ET-1, 2.6 and 1.4 fold increase eNOS, HVR Vs LVR). Among the receptors, ET_A decreased in CLP rats similarly in both the resuscitation groups whereas ET_B increased significantly as compared to sham in CLP group with HVR. iNOS mRNA level increased and HO-1 showed no change in CLP rats with no significant difference between HVR and LVR groups. The results suggest that there are alterations in the pattern of vascular gene expression in the CLP. An increase in the expression of ET-1 and eNOS demonstrate that the resuscitation amount alters the endothelial cell dependent vascular genes indicating that alteration in the shear stress due to the changes in the hematocrit may contribute to the vascular gene expression and the state of microcirculation in sepsis. *Supported by DK38201 and the Foundation for the Carolinas.*

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Deficiency In CD8 T-Lymphocytes Compromises the Ability of Mice to Survive Sepsis.

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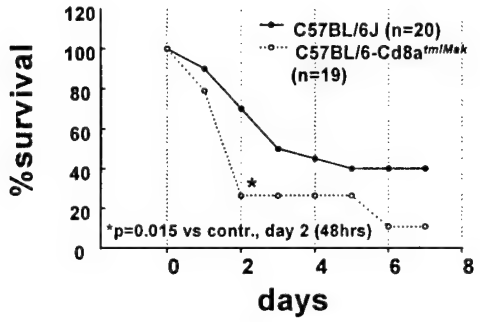
It is known that the gut associated lymphoreticular tissue in parafollicular regions are enriched with unique T-cell populations that play a crucial role in maintenance of gut function. Furthermore, the majority of studies looking at immune dysfunction following shock or sepsis have looked primarily at the contribution of peripheral CD4+ T-lymphocytes and not at the role of CD8+ T-lymphocytes lineage. Our laboratory has recently documented the marked dysfunction in the intraepithelial lymphocyte compartment of the gut, a tissue high in CD8+ and γ–δ T-cells. So, we wanted to test the hypotheses that loss or depression of the CD8+ T-cell population compromises the animal's ability to ward of septic challenge. To do this six-

week-old male *C57BL/6Cd8a^{tm1Mak}* (CD8 deficiency) and *C57BL/6J* (control) were subjected to cecal ligation and puncture and their survival monitored for 7 days.



The results indicate that mice deficient in functional CD8+ T-cells show a marked (*p=0.015, Fisher's Exact test) decline in their ability to ward of the lethal effects of septic challenge. This implies that CD8+ T-cells in the gut or other peripheral sites actively contribute to the maintenance of host defense during polymicrobial sepsis.(Supported by NIH GM 53209)

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L-ARGININE STABILIZES IκB-α AND PREVENTS CHEMOKINE PRODUCTION IN LPS INDUCED ACUTE LUNG INJURY CM Calkins*, JK Heimbach*, X Meng*, DD Bensard, BD Shames*, L Ao*, B Pomerantz, RC McIntyre
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Chemokines such as CINC-1 and MIP-2 are a group of chemotactic cytokines which stimulate the influx of leukocytes into tissues. Chemokine production is stimulated by NFκB – an inducible transcription factor which is regulated by IκB-α. We have previously demonstrated that the substrate for NO synthesis, L-arginine (L-arg), attenuates neutrophil accumulation in LPS induced lung injury. We **hypothesized** that L-arginine would attenuate the production of lung chemokines by stabilizing IκB-α and preventing the DNA binding of NFκB. The **purpose** of this study was to examine the effect of L-arg on the transcriptional regulation of chemokine production in vivo. **Methods:** Rats were injected IP with saline, D or L-arg (300 mg/kg IP) 30 min prior to LPS (0.5 mg/kg IP). In a separate group of rats, a selective iNOS inhibitor (AMT) was given 30 min prior to arginine. Lungs were excised at 2 hr following LPS for determination of CINC and MIP2 protein (ELISA), mRNA (Northern Blotting), IκB-α (Immunoblotting) and NFκB DNA binding (EMSA). **Results:** LPS induced the production of CINC-1 and MIP-2 protein and mRNA. L-arg (but not D-arg) attenuated the production of CINC and MIP-2 protein and mRNA, prevented the degradation of IκB-α, and inhibited NFκB DNA binding. Selective iNOS inhibition prevented the L-arg induced attenuation of chemokine production. **Conclusions:** L-arginine inhibits chemokine protein and mRNA production in rat lung following LPS. This effect is associated with stabilization of IκB-α and inhibition of NFκB DNA binding and appears to be a mechanism for attenuation of neutrophil accumulation in the lung following LPS.

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IDENTIFICATION OF GENES MODULATING THE EXPRESSION OF TUMOR NECROSIS FACTOR α IN MICE DURING ENDOTOXEMIA.
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A genetic approach was used to identify genes involved in the inflammatory response induced by the administration of *E. coli* lipopolysaccharide (LPS). Cytokine plasma levels, particularly tumor necrosis factor-α (TNF-α), were observed to be different between two inbred strains of mice, A/J and C57BL/6J (B6) after intraperitoneal (IP) injection of LPS. These two strains of mice are founders of a colony of recombinant inbred strains of mice (RIs). RIs were derived by inbreeding B6 and A/J mice over 20 generations to produce several new inbred strains, each with a different combination of alleles from the founder strains fixed to homozygosity. The analysis of these RIs allows us to identify the contributing genes to the TNF-α phenotype. AXB and BXA RIs were injected IP with LPS (15 mg/kg) and blood samples were collected via cardiac puncture 1.5 h post injection. TNF-α plasma levels were measured using a commercial ELISA. RI strains showed three phenotypes: high responders (like A/J), low responders (like B6), and intermediate responders. Analysis of quantitative trait loci by Map Manager QT revealed significant linkage on mouse chromosome 9. This locus spans 4 cM and contains several possible candidate genes, including the interleukin 10 receptor. This result indicates the feasibility of using mouse genetics to identify genes that contribute to the inflammatory response initiated by endotoxin.
Supported by NIH grant GM57317 and the Robert Garrett Research Foundation.

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CHRONIC SEPSIS INITIALLY MODULATES PHENYLEPHRINE-INDUCED CONTRACTION BY ALTERING NITRIC OXIDE SYNTHASE 1+3 (NOS 1+3) ACTIVITY. R. N. Garrison, T. Kawabe, P. D. Harris, and D. A. Spain. Dept Surgery & Ctr Applied Microcirculatory Res, Univ. Louisville & VAMC Louisville KY 40292.

Phenylephrine (PE), a selective alpha-1 adrenergic receptor agonist, is a vasoconstrictor, but PE also increases Nitric Oxide (NO) release to limit PE-induced contraction as protection against vessel occlusion. Our study asked if chronic sepsis modulates agonist-induced contractions by altering this secondary NO release. Chronic sepsis was induced by inoculation (E.Coli and B. Frag) of subcutaneous sponges. Septic(n=8) and saline(n=9) rats were studied at 24hrs after a single inoculation. Other rats (72hr, n=18) were studied after a 2nd inoculation 48hrs later. Aortic rings (2mm) from each rat were put on 1g preload in baths filled with PSS or PSS+inhibitor (L-NNA for NOS 1+3; L-NMMA for NOS1+2+3 inhibition). Rings were PE and acetylcholine pretreated; washed; then contracted with 6 doses of PE (0.01 - 3uM). The maximum contraction (Fmax) to PE was greater in 24hr sepsis than in 24hr saline, 72hr sepsis, and 72hr saline rats (Table 1). Both L-NNA and L-NMMA had less effect on Fmax in 24hr sepsis than in 24hr saline, 72hr sepsis, and 72hr saline rats (Table 2).

TABLE 1.	24hrSep		24hrSal		72hrSep		72hrSal	sem
Fmax(g)	1.15	>	0.77	=	0.68	=	0.67	0.072

TABLE 2.	24hrSep		24hrSal		72hrSep		72hrSal	sem
+ΔF(g) LNNA	0.59	<	0.93	=	0.96	=	0.83	0.047
+ΔF(g)LNMMA	0.82	<	1.20	=	1.19	=	1.14	0.029

We conclude that PE increases NOS 1+3 activity. This NOS 1+3 effect is decreased early in sepsis (24hr) but this decrease is reversed later in chronic (72hr) sepsis. (Funded: CAMR, VA Merit, US Dept Defense)

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INCREASED PRELOAD ENHANCES SUPPRESSION OF PHENYLEPHRINE (PE)-MEDIATED INCREASES IN NITRIC OXIDE SYNTHASE (NOS 1+3) ACTIVITY DURING CHRONIC SEPSIS. T. Kawabe, P. D. Harris, M. A. Wilson, and R. N. Garrison. Ctr Appl Microcirc Res & Dept Surgery, Univ. Louisville & VAMC, KY 40292.

PE increases NOS 1+3 activity to limit PE-induced contraction at low preload (PL) but less so at high PL. 24hr sepsis enhances PE contractions by decreasing PE-induced increases in NOS 1+3 at low PL. Our study asked if chronic sepsis has any effect on PE-induced contraction at higher PL. Chronic sepsis was induced by inoculation (E. Coli and B. Frag) of subcutaneous sponges. Septic (n=8) and saline (n=9) rats were studied at 24hrs after a single inoculation. Other rats (72hr, n=18) were studied at 72 hr after two inoculations 48hrs apart. Aortic rings (2mm) were put on 5g PL in baths filled with PSS or PSS+inhibitor (L-NNA for NOS 1+3; L-NMMA for NOS 1+2+3 inhibition). All rings were agonist pretreated; washed; and contracted with 6 doses of PE (0.01 - 3uM). The max contraction (Fmax) to PE without NOS inhibitor was greater in 24hr sepsis; but, Fmax with NOS inhibitor was less in 24hr sepsis than in the other groups (Table). NOS inhibitor had no effect on PE contraction in 24hr sepsis; yet, PE contraction without NOS inhibitor in 24hr sepsis (2.31) was less than with NOS inhibitor in 24hr saline (2.66 & 2.77) (Table).

TABLE	24hrSep		24hrSal		72hrSep		72hrSal	sem
Fmax(g) wo INHIB	2.31	>	1.90	=	1.68	=	1.57	0.011
Fmax(g) L-NNA	2.33	<	2.66	=	2.73	=	2.60	0.091
Fmax(g) L-NMMA	2.33	<	2.77	=	2.85	=	2.65	0.080

We conclude that 1) PE-induced NOS 1+3 activity is abolished at higher PL in 24hr sepsis; 2) PE increases a non-NOS dilator at higher PL in 24hr sepsis; but this non-NOS PE effect returns to normal at 72hr of sepsis. (Funded: CAMR, VA Merit, US Dept Defense)

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OVEREXPRESSION OF HIGH-AFFINITY Fcγ RECEPTOR (CD64) ON LEUKOCYTES IN SEPSIS. Mark Hirsh, Eugenia Mahamid, Yulia Bashenko, Irina Hirsh and Michael M. Krausz. Dept of SurgeryRambam Medical Center, Carmel Medical Center,

An increase in CD64+ monocytes has been demonstrated in septic patients, and an association between cell number, activity and poor outcome was described. In the present investigation further characterization of CD64+ leukocytes has been attempted. Twenty-three patients with a major septic episode were compared to a control group of ten healthy volunteers. Flow cytometric analysis of surface leukocyte antigens, phagocytosis and reactive oxygen metabolites (ROM) production was performed.
Results: CD64 expression on monocytes (Mo) and granulocytes (Gra) was markedly increased in septic patients ,and even more in sepsis with ARDS. In healthy individuals phagocytic activity (PA) of CD64+ and CD64– Mo was similar, while phagocytic activity (PA) of CD64+ Gra was higher, than of CD64– cells. In septic patients decreased PA was detected in CD64+ Mo and Gra. CD64+ Gra of patients in sepsis and ARDS exhibited the most prominent PA depression. ROM production in unfractionated Mo and Gra was increased in sepsis. No additional increase in ROM output was detected in ARDS. In healthy individuals CD64+ Gra and stimulated CD64+ Mo demonstrated increased ROM synthesis compared to matched CD64– cells. ROM production by CD64+ leukocytes in sepsis was also increased compared to CD64– cells. CD64+ leukocytes of septic patients generated significantly less ROM compared to healthy subjects.
Conclusion: over-expression of CD64 on Mo and Gra in sepsis and ARDS is associated with decreased phagocytic activity and decreased oxidative response.

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cDNA Array Screening Reveals Corticosteroid Inhibition of Early Transcriptional Responses to Endotoxin. MR Lerner, LM Landrum*, ER Jupe*, JS Hanas,*RG Postier,* DJ Brackett, Depts. of Surgery and Biochemistry & Molecular Biology, Univ. Oklahoma HSC; VA Medical Center; & Okla. Med. Res. Found. Okla. City,OK

Prophylactic high dose corticosteroids prevent endotoxin (ETX)-induced lethality and increase survival even when given after ETX. ETX challenge evokes significant physiological changes within minutes of its intravenous administration. We have applied cDNA array methodology (Clontech) to evaluate the effect of Methylprednisolone (MP) on the initial expression responses of 588 genes (known functions) to ETX. Male rats were given intravenous *1)* saline, *2)* MP, 300mg/kg, *3)* ETX, 20 mg/kg (LD_{100}-24 hrs), *4)* MP 15 min prior to ETX. Hepatic tissue was harvested 10 min after ETX and RNA was prepared for hybridization to the cDNA array, phosphorimaging, and computer analysis (Imaging Research). MP prevented 60% of transcriptional responses (up- and down-regulation) induced by ETX. These genes were associated with protein turnover, lipid & steroid metabolism, and channels & transporters. MP alone evoked no changes; however, in combination with ETX significant up-regulation was observed in genes associated with protein turnover, cell-cell comunication, and channels & transporters. The capacity of MP to prevent lethality may be related not only to inhibition of

early transcriptional activity induced by ETX, but also to its significant up-regulation of gene activity that occurs only in the presence of ETX. Parallel, large-scale screening of genetic transcriptional activity in models relative to sepsis may reveal new insight and identify interventional targets relative to therapeutic strategies.

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PRO-INFLAMMATORY CYTOKINES AND FIBROSIS ARE INDUCED IN SEPTIC RAT LUNGS BY INTRA-ABDOMINAL ABSCESS FORMATION, LEADING TO ARDS-LIKE ABNORMALITIES. J.R. Lussier, N.G. Espina, and J.H. Siegel, UMDNJ-New Jersey Medical School & Graduate School of Biomedical Sciences, Newark, NJ 07103.

The onset of a septic condition accounts for more than one third of acute respiratory distress syndrome (ARDS) cases, of which the most prevalent sources are lung and intra-abdominal infections. In order to study the ARDS-like changes that occur as a result of intra-abdominal abscess formation, we utilized a septic rat model, in which a 1.5cc fecal-agar pellet, either sterile or contaminated with 10^2 *E.coli* and 10^8 *B.fragilis*, was implanted into the peritoneal cavity of a rat. Pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), fibrinogen, and collagen (Type III) gene expression (mRNA) was demonstrated by *in situ* hybridization, in abscess and lung tissues at Day 3. Gene expression of all mRNA's was higher in the septic than in the sterile abscesses, and corresponded in time to the increased right heart plasma levels of the cytokines. After abscess formation at Day 3, histologic examination of lung tissue was carried out with routine H&E and Gomori's trichrome stains. The pattern of ARDS was evident in the lung as seen by cellular infiltration, increased interstitial connective tissue deposition, and decreased compliance. In the septic lung macrophages (mϕ), IL-6 and TNF-α mRNA was seen to be elevated above control levels. The sterile lung tissue mRNA levels were increased above control, but were not at the same magnitude as in the septic rat. IL-1β was not expressed in the lung tissues at Day3. Fibrinogen and collagen III gene expression was demonstrated in lung fibroblasts to be higher in the sterile than in control, but not as high as in the septic. We propose that the formation of an intra-abdominal abscess releases circulating cytokines, which in turn activates lung mϕ's to produce these pro-inflammatory cytokines. Then the cytokines induce local fibrinogen and collagen synthesis in lung tissue, leading to ARDS-like changes.

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A ROLE FOR THE ABDOMINAL VAGUS IN LIPOPOLYSACCHARIDE (LPS) - INDUCED HYPOTENSION. D. Mailman, Univ. Houston, Houston, TX 77204.

LPS - induced hypotension may be mediated through nitric oxide (NO) and activated leukocyte-derived cytokines. I investigated whether the abdominal vagus also affected LPS- induced hypotension, analogous to the role of the afferent vagus in LPS - induced fever. Female rats were anesthetized with Nembutal. The subdiaphragmatic vagal trunk was suffused with 2% Lidocaine or saline over gauze packing. LPS (5 mg/kg) or saline were injected i.v and their effects followed for 3 hr. Femoral arterial blood pressure was measured. A leukocrit was determined from the thickness of the buffy coat measured by microscopic micrometry in capillary tubes. NO3- plus NO2- (NOx) were measured after NO3- reduction and the Griess reaction. LPS - induced hypotension was significantly attenuated by vagal Lidocaine. The LPS - induced increase in plasma [NOx] and the decrease in leukocrit were not affected by vagal anesthesia. I.v. saline plus vagal lidocaine did not change blood pressure, plasma [NOx] or leukocrit. The surgery required to place the gauze, as compared to laparotomy alone, had no significant effects. It was concluded that the abdominal vagus is an important component of the early LPS-induced hypotension, along with NO and leukocyte activation. Whether the vagal fibers were afferent and/or efferent was not determined.

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REGULATION OF GLUCOSE-6-PHOSPHATASE GENE EXPRESSION IN SEPSIS. Subir R. Maitra, Rafaat El-Maghrabi, Collin E. M. Brathwaite. Trauma Research Lab, Depts of Emerg Med and Surg, Univ Hosp and Med Ctr, SUNY, Stony Brook, NY 11794.

The glycemic response to sepsis is largely, but not exclusively, the result of changes in the rate of glucose production by the liver. The increased glucose production that occurs during the early hyperglycemic phase of sepsis is not maintained and the latter hypoglycemic phase is characterized by either a relative or absolute depression in gluconeogenesis. The molecular mechanisms underlying these changes in glucose homeostasis, however, are not well defined and are the focus of this study. Sepsis was induced in anesthetized, fasted rats by cecal ligation and puncture (CLP), and liver samples were taken at 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 5 h, and 20 h post CLP. The mRNA abundance of hepatic Glu-6-Pase increased 4-fold at 0.5 h compared to control values, 2-fold after 1 h, and returned to normal after 1.5 h. This was followed by a corresponding increase in Glu-6-Pase activity, and was coincident with increased plasma glucose levels and decreased liver glucose-6-phosphate (Glu-6-P) at 0.5 h and 1 h. Plasma insulin and glucagon levels remained unchanged during this period, while corticosterone levels increased 2.5-fold over control values. At 20 h CLP, plasma glucose levels returned to normal, coincident with 90% reduction in Glu-6-Pase mRNA abundance. Glu-6-Pase activity and Glu-6-P concentration returned to normal levels while insulin, glucagon and corticosterone levels increased significantly compared to time zero control. Our data indicate that the initial rise and subsequent decline in blood glucose correlate very well with a corticoster-

one-dependent induction of hepatic Glu-6-Pase, mRNA and protein, followed by an insulin-dependent suppression of its expression. (Supported by NIH GM 58047 and GM 52025)

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NAFAMOSTAT ATTENUATES SHOCK AND LUNG INJURY IN SEPSIS FOLLOWING SMOKE INHALATION IN SHEEP

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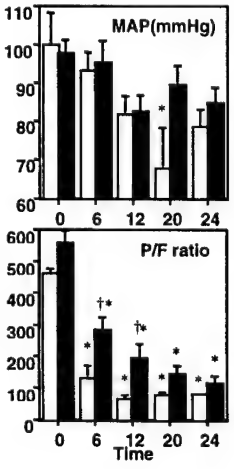
The lesions of smoke inhalation often progress into sepsis and acute respiratory distress syndrome. Nafamostat mesilate (NM) is a synthetic serine protease inhibitor, which inhibits thrombin, factor Xa, factor VIIa, etc. The aim of this study was to clarify the effect of NM on sepsis after smoke inhalation in sheep. Merino ewes (n=8) were surgically prepared for the study. Five to seven days later, animals received a tracheostomy and 48 breaths of cotton smoke. *Pseudomonas aeruginosa* was suspended in 30 mL saline, which contains 2-5 x 10^{11}cfu, injected into the right lobe (20mL) and into the left lobe (10mL) using a bronchoscope. After the bacterial challenge, the animals were ventilated mechanically with 100% O_2. NM (n=4) or lactate Ringer (n=4) was infused continuously from 1 h after the bacterial challenge through 24h. The sheep showed a systemic hypotension and fall in PaO_2 (FIG). These changes were attenuated by NM. NM also inhibited the intravascular fibrin formation significantly. **CONCLUSION**: MN may inhibit septic changes by inhibiting coagulation abnormalities. **FIG**: Changes in mean arterial pressure (MAP) and PaO_2/FiO_2 (P/F) ratio after smoke + bacterial challenge in NM (n=4; solid bar) and control (n=4; open bar) group. Data represents the mean ± SE. * p<.05 vs. base line and † p<.05 vs. control.



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EARLY SURGICAL INTERVENTION REDUCES MORTALITY IN A MODEL OF SEPTIC PERITONITIS. J.A. Nemzek, G. L. Bolgos*, D.G. Remick, Dept. of Pathology, University of Michigan, Ann Arbor, MI 48109.

The effects of removal of the septic focus on survival were examined using a cecal ligation and puncture (CLP) model. With animal care and use approval, female BALB/c mice were subjected to CLP with an 18 gauge needle. The mice were randomized into three groups. One group, euthanized at either 8 (n=7) or 15 (n= 7) hours after CLP, was used to obtain plasma and peritoneal lavage samples. In another group, the animals were re-anesthetized at either 8 (n=11) or 15 (n=11) hours and underwent resection of the cecum. After resection, the abdomen was lavaged and the retrieved fluid was saved for further analysis. A control group underwent CLP (n=8) but received no further surgical intervention. All animals received antibiotics and fluids in the form of subcutaneous imipenem in dextrose, twice daily, for up to three days after the CLP. The inflammatory response was investigated by measuring cytokine levels to determine if delaying surgical intervention would promote a hyperinflammatory state. Plasma IL-6 levels were significantly higher ($p<0.05$) in the 15 hour euthanasia group (21 ± 4 ng/ml) versus the 8 hour euthanasia group (9 ± 2 ng/ml). However, there was no significant difference in the local peritoneal levels of IL-6. In addition, there were no significant differences in the plasma or peritoneal lavage levels of the murine chemokines KC and MIP-2α between the 8 and 15 hour groups. Over a three-day period after CLP, the mortality rates were significantly higher ($p<0.005$) in the control (7/8) and the 15 hour resection (10/11) groups than in the 8 hour resection group (6/11). Survivors in the 8 hour resection group were followed for 7 days at which point activity levels and weight were returning to normal. Although many factors may affect the treatment of sepsis, this study would suggest that surgical intervention promotes survival when performed early, prior to overwhelming systemic inflammation.

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OXYGEN THERAPY CAUSES REDISTRIBUTION OF BLOOD FLOW TO THE SPLANCHNIC VASCULAR BED IN SEVERE SEPSIS. G. Weber*, V. Brod* and H. Bitterman. Carmel Medical Center, Rappaport Institute for Research, Faculty of Medicine, Technion, Haifa 34362, Israel.

Inhalation of oxygen is routinely used in severe sepsis and septic shock. We have previously shown that in hemorrhaged rats inhalation of 100% oxygen induces a shift of blood flow from skeletal muscles to the renal and splanchnic vascular beds. This study evaluated hemodynamic effects of oxygen in severe sepsis induced in rats by cecal ligation and puncture (CLP). All CLP rats exhibited positive blood cultures. All blood cultures in sham rats were negative. Twenty hours after CLP or sham operation we started monitoring mean arterial blood pressure (MABP), superior mesenteric artery (SMA) flow and blood flow in the distal portion of the descending aorta (DA). We also followed regional splanchnic and hindquarter vascular resistance. At that time (20 hrs) blood flow in the DA and SMA was significantly lower and hindqarter and splanchnic regional vascular resistances were significantly higher than in sham rats ($P<0.05$ or less). Inhalation of 100% oxygen induced a small but significant increase in MABP ($P<0.001$). Oxygen did not change DA blood flow or resistance in the hindquarter. In contrast, oxygen caused a significant 15±5% decrease in SMA resistance ($P<0.03$) and a 26±9% increase in SMA flow ($P<0.01$). Cessation of oxygen was followed by reversal of all hamodynamic changes. Our data suggest that redistribution of blood flow to the splanchnic vascular bed may underlie beneficial effects of oxygen in severe sepsis.

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ADMINISTRATION OF HUMAN INTER-α-TRYPSIN INHIBITOR (ITI) ATTENUATES HYPODYNAMIC RESPONSE AND LIVER INJURY DURING LATE SEPSIS. SL Yang*, YP Lim*, H Schwinn*, D Josic*, IH Chaudry, and P Wang. Brown University School of Medicine and Rhode Island Hospital, Providence, Rhode Island 02903 and Octapharma Pharmaceuticals, Vienna, Austria.

Although studies have shown that plasma ITI decreases in septic patients, it remains unknown whether administration of ITI early after the onset of sepsis has any beneficial effects on cardiac output (CO), O_2 utilization, and hepatocyte integrity. To determine this, sepsis in male adult Sprague-Dawley rats was induced by cecal ligation and puncture (CLP) followed by fluid resuscitation. At 1 h after CLP, ITI at a dose of 30 mg/kg BW or normal saline (NS, 1 ml/rat) was infused IV for 30 min. At 20 h after CLP (i.e., the late stage of sepsis), CO (ml/min/100 g BW) was measured using a dye dilution technique. Blood samples were collected for determining O_2 content, and O_2 delivery (DO_2, ml/min/100 g BW), O_2 consumption (VO_2, ml/min/100 g BW) and O_2 extraction ratio (O_2ER) were then calculated. Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), lactate, and TNF-α were also measured. The results (mean ± SE, n=6/group) are as follows:

	Sham	CLP+NS	CLP+ITI
CO	37.7±2.1	24.1±1.9*	33.2±1.6#
Systemic DO_2	7.3±0.2	5.4±0.5*	7.4±0.3#
Systemic VO_2	2.7±0.3	2.5±0.3	3.9±0.2*#
Systemic O_2ER (%)	36.6±3.2	46.1±3.1	52.9±0.8*
ALT (IU/L)	6±2	60±2*	28±2*#
AST (IU/L)	45±3	135±3*	91±5*#
Lactate (mg/dL)	15±1	69±2*	49±1*#
TNF-α (pg/ml)	13±13	532±202*	62±25#

(ANOVA and Tukey: *P<0.05 vs Sham; #P<0.05 vs CLP+NS)

The results indicate that the administration of ITI at 1 h after CLP maintained CO and systemic DO_2, increased systemic VO_2 and O_2ER. Moreover, ITI downregulated TNF-α production and attenuated hepatocellular injury and lactic acidosis at 20 h after CLP. Thus, ITI appears to be a useful adjunct for maintaining hemodynamic stability and preventing organ injury during the progression of polymicrobial sepsis.

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LUNG COMPARTMENTALIZATION OF MONONUCLEAR CELLS FOLLOWING CLP. K. Yin*, J. Wilmanski*, C. Wang*, G. Qiu* and M. Tahamont*. (Spon: C. Hock). UMDNJ-SOM, Stratford, NJ 08084 and Rowan University, Glassboro, NJ 08028.

Lung injury commonly occurs in the setting of systemic inflammatory response syndrome (SIRS) occurring during bacterial sepsis. We examined the pathogenesis of lung injury after cecal ligation and puncture (CLP), a clinically relevant model of sepsis. At 24h post CLP, histological analyses showed that there were signs of edema in the lung, while at 48h after CLP, alveolar wall thickening with increased cellularity and diffuse alveolar hemorrhage was clearly observed. To assess the sequestration and migration of leukocytes, differentials were obtained for the lung vascular compartment and the bronchoalveolar airspace. The number of lymphocytes in the pulmonary vascular compartment dropped by 50% and doubled in the BAL, 24h after CLP compared to sham controls suggesting that there was transendothelial migration. At 48h after CLP, lymphocyte numbers in the lung vasculature was similar to controls but BAL lymphocytes were still raised. The number of pulmonary intravascular neutrophils were similar to controls at 24h post CLP but were elevated by 3.5 fold, 48h after CLP. The increase in neutrophils was partly due to a substantial increase in immature band cells, indicating recruitment of neutrophils from the bone marrow. There were very few neutrophils in the BAL of controls and CLP rats. Perfusate monocyte/macrophages were increased by 3-fold, 48h after CLP and a similar increase in macrophages was observed in the BAL. These results strongly suggest a role for lymphocytes and macrophages in the development of overt lung injury in CLP sepsis as migration of these cells corresponds to the appearance of lung injury. With respect to this, our data demonstrates the compartmentalization and migration of different inflammatory cell-types in the lung during the development of sepsis post CLP.

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ADENOVIRAL-MEDIATED OVEREXPRESSION OF INHIBITORY KAPPA-B ALPHA DOES NOT IMPROVE SURVIVAL OF SEPTIC RATS. Toshihiko Mayumi[1], Shuji Hayashi[2]*, Jun Takezawa[1]*. [1]Department of Emergency Medicine and Intensive Care Unit, [2]Second Department of Surgery, Nagoya University School of Medicine, Nagoya, 466-8560, Japan

Background and Objective: In sepsis, phosphorylation and degradation of inhibitory kappaB-alpha (IkB-α) occurred which leads nuclear factor-kappaB (NF-κB) to activate proinflammatory cytokine genes (TNF-α, IL-1, IL-6, etc) transcription. Here we investigated whether overexpression of IkB-α provided by adenoviral gene transfer could prevent survival of panperitonitis in rats.

Materials & Methods: Adenovirus (Adex/IkB-α) which transfers IkB-α gene and produces IkB-α was made. Adex/LacZ that transfers β-galactosidase gene was used as control. Study 1: One hour after cecum ligation and puncture (CLP), Adex/LacZ(n=8) or Adex/IkB-α(n=8) ($1.3\text{-}2.0\times10^8$PFU/ml) was injected into the spleen of male Wistar rats. Plasma IL-6 and TNF α levels at 24 hours after CLP and the survival rate were examined. Study 2: One hour after CLP, Adex/LacZ(n=8) or Adex/IkB-α (n=8) ($3.9\text{-}11.2\times10^8$PFU/ml) was injected into abdominal cavity of male Wistar rats. The plasma IL-6 and TNF α levels at 24 and 48 hours after CLP and the survival rate were examined.

Results: IkB-α was over-produced during 1 to 3 days after Adex/IkB-α injection. Study 1: Mean and median survivals of IkB-α were 6.6 and 6 days whereas those of Adex/LacZ were 7.6 and 7.5 days (no significant difference). Study 2: Mean and median survivals of IkB-α were 11.5 and 7 days vs. those of Adex/LacZ were 16.5 and 15 days (no significant difference).

Conclusions: Overexpression of IkB-α by adenoviral gene transfer does not improve survival of septic rats.

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IS THE DEPRESSION OF SPLENIC T LYMPHOCYTE FUNCTIONS FOLLOWING TRAUMA-HEMORRHAGE (TH) DUE TO CHANGE IN STEROIDOGENIC ENZYME ACTIVITIES? T.S.A. Samy*, M. W. Knöferl, M. G. Schwacha, K. I. Bland* and I. H. Chaudry. Center for Surg. Res., Brown Univ. Sch. of Med., Rhode Island Hosp., Providence, RI 02903.

5α-reductase, aromatase and 3β- and 17β-hydroxysteroid dehydrogenases (3β-HSD,17β-HSD), the enzymes involved in sex

steroid metabolism, are present in the spleen. However, it is not known whether these enzymes play an important role in the local production of dihydrotestosterone and β-estradiol and whether they are related to the loss of T cell functions seen in males, but not in females following TH. TH was induced in male and female mice by soft-tissue trauma (laparotomy) and hemorrhage (30mmHg arterial pressure, 90 min) followed by resuscitation with 4x Ringer's lactate. After 24h, adrenals, gonads and spleen were removed, splenic T cells prepared and assayed for the above enzyme activities. 5α-reductase activity was higher in testes and aromatase activity in the ovary (Table).

	5α-Reductase **		Aromatase **	
	Male	Female	Male	Female
Testes S	120.0 ± 5.7		2.7 ± 0.7	
TH	165.3± 11.2*		3.3 ± 1.1	
Ovary S		0.7 ± 0.4		30.4 ± 5.1
TH		0.8 ± 0.3		69.5 ±6.8*
T cells S	47.8 ± 13.3	17.0 ± 2.3	5.0 ± 0.6	4.0 ± 2.4
TH	130.0± 11.2*	18.6 ± 3.6	6.3 ± 0.3	12.9 ± 2.1*

S, Sham; TH, Trauma-hemorrhage; * P< 0.05. ** pmol/mg protein/min

TH significantly increased the activity of both the enzymes; the increase following TH was 2-3 fold more in splenic T cells than in gonads or adrenals. There was no change in the splenic 3β-HSD activity (pmol/mg protein/h) of males (S, 415±38; TH, 465±45) or females (S, 425±27; TH, 476±40). Although 17β-HSD activity (nmol/per mg protein/h) was unchanged in males (S, 0.32±0.02; TH, 0.37±0.03), it increased 5-fold in females (S, 0.46±0.12; TH, 2.4±0.28) after TH indicating β-estradiol synthesis. Increased activity of 5α-reductase in male splenic T cells following TH should result in increased production of dihydrotestosterone for receptor binding and activation, thus, leading to depressed T cell functions. (Supported by NIH grant GM37127.)

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MATHEMATICAL MODELS OF HOST RESPONSE TO BACTERIAL CHALLENGE, Robert L. Fulton, VA Medical Center, 800 Zorn Avenue, Louisville, Kentucky 40206-1499.

Despite intense investigation, qualitative descriptions of host response to bacterial challenge (HR-BC) are enigmatic. Different responses to infection occur between the best-controlled experiments and seemingly alike patients. Consistent effective treatment for sepsis, MSOF and SIR is not available. Like other natural systems, HR-BC can be examined mathematically. The reasons to attempt the modeling are to gain clearer understanding of the phenomenon, and to promote more effective manipulations of the disease process. Systems of differential equations (DE's) were developed which show interrelationships of HR-BC. To be useful, the model must produce infection which is quickly eradicated, persists for some time or overwhelms the host. A temporal quantitative relationship of HR to BC must exist (closed loop feedback). External treatment and development of bacterial resistance need to be included in the analysis. Basically,

$$\frac{dB}{dt} = b_0 B(t) - b_n(B(t),H(t)) \qquad \frac{dH}{dt} = f(B(t), \sum h_n(t); a_n)$$

$$\frac{dh_n}{dt} = f(B(t),h_n;d_n) \qquad \frac{dT}{dt} = f(B(t),T(t);c_n)$$

$$\frac{dI}{dt} = f(\Sigma h_n; m_n)$$

Where dB/dt, dH/dt, dT/dt and dI/dt are the changes in time of bacteria, total host response, bacterial toxins and inflammatory agents, respectively. Σhn is the sum of HR's cellular and humeral agents. Rate coefficients are b_n, a_n, c_n and m_n. Graphic solutions are obtained numerically. Altering coefficients produce a family of solutions which model eradication of infection, overwhelming infection and persistence of inflammation, even after the bacteria have disappeared. Development of resistance and treatment schedules can be incorporated into the system of DE's. Stable, unstable and chaotic solutions which mimic experimental descriptions of infection are obtained. The analysis indicates a) why conflicting results occur in HR-BC research, b) why some treatments "work" and why some fail, and c) what research in HR-BC must be performed to quantitatively relate the various factors of HR-BC to each other.

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ENDOGENOUS ADENOSINE: A PLURISYSTEM MODULATOR DURING SEPSIS William R. Law
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Our laboratory has hypothesized that during sepsis, endogenous adenosine plays key roles in vascular, immunological, and oxyradical-mediated events. The present study was undertaken to determine if modulation of endogenous adenosine by pharmacologically distinct methods would significantly alter tissue concentrations of TNF-α, and oxidative damage [as measured by thiobarbituric acid reactive substances (TBARS)]. Rats were made septic by IP injection of 200 mg/kg cecal slurry (in 5 ml/kg D5W). Nonseptic (sham sepsis) controls received 5 ml/kg D5W. On the day of sepsis/sham sepsis induction, rats received IP saline (untreated), 20 mg/kg 8-sulfophenyltheophylline (8-SPT; adenosine receptor antagonist), or 5 mg/kg pentostatin (PST; adenosine deaminase inhibitor). Twenty-four hours later, animals were euthanized to obtain plasma and tissue samples for measurement of TNF-α (ELISA) and TBARS (colorimetric). Statistical analysis was by analysis of variance followed by least significant difference (LSD) test ($p<0.05$). Untreated sepsis resulted in significantly higher concentrations of TNF-α in liver (91 ± 13 ng/g tissue) and TBARS in jejunem (5.3 ± 0.7 nmol/mg protein), compared to non-septic rats (15 ± 9 and 2.8 ± 1.7, respectively). These responses were amplified when adenosine receptors were blocked (8 SPT group; liver TNF: 162 ± 23; jejunum TBARS: 6.9 ± 0.5), but both TNF-α and TBARS concentrations were attenuated when the degradation of endogenous adenosine was inhibited (PST group; TNF: 47 ± 12; TBARS: 3.6 ± 0.2). These data indicate that in sepsis, amplifying or negating activity of endogenous adenosine impacts upon oxidative damage and TNF-α concentrations. Because the effects of endogenous adenosine concentrations appear modulatory rather than obligatory, novel manipulation of adenosine activity in sepsis may prove useful as a therapeutic tool.

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THE ROLE OF CYTOKINES IN BONE GROWTH DYSFUNCTION AFTER POLYTRAUMA - ACTIONS ON OSTEOBLASTS IN VITRO. L. Mahlke*, H.-C. Pape, A. Seekamp*, J. Zeichen*, M. van Griensven
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Heterotopic ossifications result in functional deficits or even ankyloses. The pathophysiology of heterotopic

ossifications is not fully understood. It is expected that osteoblasts may play a role. A correlation is observed in the occurrence of heterotopic ossification in patients suffering from traumatic brain injury or polytraumatic patients undergoing long time mechanical ventilation. Increased serum levels of pro-inflammatory cytokines are detected. This lead to the question whether cytokines influence osteoblastic activity and therefore may play a role in the pathogenesis of heterotopic ossification. Osteoblastic activity was measured by means of alkaline phosphatase (AP) activity. The osteoblasts were stimulated with TNFα, IL-1β, or IL-6 for 4 hours. As a negative control, no stimulating agents were applied to the cells. Overall, AP activity was higher in the supernatants than in the cell homogenates. TNFα resulted in the highest AP activity in the supernatant (316%). TNFα did not cause an increase of AP activity in the cell homogenate (103%). IL-1β increased AP activity in cells of 203%. AP activity of IL-6 amounted to 140% in cell homogenates. TNFα and IL-6 cause an increased secretion of AP, whereas IL-1β leads to an increase in both secretion and production. Inflammatory cytokines result in an increased AP activity of osteoblasts. As these cytokines are increased in polytraumatic patients, these mediators may play a role in the pathogenesis of heterotopic ossifications.

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DOES 3-AMINOPROPANAL AFFECT SPLENOCYTE APOPTOSIS AND/OR FUNCTION? L. Watkins*, C.S. Chung, G.Y. Song*, S. Ivanova*, K.J. Tracey and A. Ayala, Brown Univ. Sch. Med./ Providence Col., Providence, RI 02903 & Picower Inst., Manhasset, NY 11030

The mechanism by which ischemic injury induces cellular damage is an area of active interest in the understanding of stroke, traumatic shock and certain aspects of sepsis. In this regard, prior studies indicate that 3-aminopropanal (3-AP), a product of spermine oxidation by polyamine oxidase released during ischemic injury, is linked with apoptosis in glial cells and necrotic neuronal cell death. Interestingly, following the onset of shock or sepsis, many cells of the immune system undergo a significant increase in apoptotic cell death associated with cell dysfunction, the mediator(s) of which remains unknown. Therefore, our specific aim was to determine if 3-AP could alter splenocyte viability, ensuing cell death and cytokine production. To do this, first, we isolated splenic macrophages and then splenic lymphocytes from normal C3H/HeN male mice. Lymphocytes were stimulated with Concanavalin A while splenic macrophages were stimulated with lipopolysaccharide, in the presence of varying concentrations of 3-AP for either 6h or 24h. Cell viability was determine by trypan blue exclusion. After 24h, the presence of apoptosis was ascertained by propidium iodide staining. Cytokine production was assessed by ELISA. The results obtained indicate that after 6h incubation with 10 μM and 100 μM concentrations of 3-AP, there is a marked decrease ($p<0.05$, Mann-Whitney U) in lymphocyte viability. Apoptosis, as determined by flow cytometry, is significantly increased among splenocytes subjected to 10 μM 3-AP. Splenocyte production of IL-10 appears to be influenced by the presence of 3-AP, but the exact effect has yet to be determined. Interestingly, splenic macrophages are less sensitive to 3-AP, as their viability is not affected by concentrations of 3-AP up to 10 μM. These findings suggest that 3-AP has divergent effects on lymphoid/ macrophage cell death and function, which may play a role in immune dysfunction, seen in shock and septic states. (Supported by NIH GM53209 & GM57226)

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INDUCTION AND EXPRESSION OF β-CALCITONIN GENE-RELATED PEPTIDE IN RAT T LYMPHOCYTES AND ITS SIGNIFICANCE. L.Xing*, J. Guo* and X.Wang. Inst. Vas. Med., Third Hos., Beijing Med. Univ., Beijing 100083, P.R. China.

Our previous data have shown that rat lymphocytes can synthesize calcitonin gene-related peptide (CGRP), a 37 amino acids neuropeptide. In this study, the type, the characteristics and the functional role of lymphocyte-derived CGRP were investigated. The results showed that Con A and rhIL-2 induced CGRP synthesis and secretion by lymphocytes from both thymus and mesenteric lymph nodes in a time-dependent manner. Treated with Con A 4 μg/ml or rhIL-2 750 U/ml, the CGRP secretion in both cells was raised significantly at 3 days and increased further at five days. Stimulation of these cells with Con A (1-8 μg/ml) for five days induced a significant increase of CGRP secretion in a concentration-dependent manner. The maximal increases of CGRP synthesis in two sources of lymphocytes were 132.2% and 127.3% above controls, respectively. The maximal secretions of CGRP by both cells were elevated from 104.0±10.8 pg to 381.3±43.8 pg/10^8 cells, and from 83.0±10.3 pg to 348.7±25.4 pg/10^8 cells, respectively. Treatment with rhIL-2 (94-1500U/ml) for five days also induced a significant increase of CGRP secretion by both cells in a concentration-dependent manner. The maximal CGRP secretion by both cells was elevated to a highest levels of 607.3±23.0 pg/10^8 and 704.0±36.8pg/10^8 cells, respectively. The nucleotide sequencing study showed that lymphoid cells expressed β-CGRP cDNA only. The levels of β-CGRP mRNA in mitogen-stimulated lymphocytes of both sources were also increased. However, LPS had no effect on β-CGRP synthesis and secretion from both source cells. $hCGRP_{8-37}$ (2.0 μM), a $CGRP_1$ receptor antagonist, enhanced Con A-induced proliferation and IL-2 release of thymocytes significantly. The data suggest that production of endogenous β-CGRP of lymphocytes can be induced by T lymphocyte mitogens. The immune system is another source of β-CGRP that may inhibit partially the proliferation and IL-2 release of rat T lymphocyte under immune challenges.

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AGENT-BASED COMPUTER SIMLUATION AND SIRS/MODS: BUILDING A BRIDGE BETWEEN BASIC SCIENCE AND CLINICAL TRIALS. G. An* and K. Nagy. Cook County Hospital, Chicago, IL 60612

The management of Systemic Inflammatory Response Syndrome (SIRS)/Multi-Organ Dysfunction Syndrome (MODS) remains the greatest challenge in the field of critical care. There has been uniform difficulty in translating basic science knowledge into effective therapeutic regimes. We propose that this be due to a failure to account for the complex, nonlinear

nature of the inflammatory process, of which SIRS/MODS represents a disordered state. It is extremely unlikely that there exists one key metabolic step to target. Therefore formulating therapies will require the ability to model multiple concurrent variables. Attempts to manipulate this process without a dynamic overview of the system are bound to produce unintended consequences. Agent-Based Computer Simulation (ABCS) provides a means by which the information acquired from the linear analysis of basic science can be reconstructed into a model that preserves the complexity of the inflammatory system. We have constructed an abstracted version of the inflammatory process using an ABCS that is based at the cellular level. The simulation space is the interface between the endothelial lining and blood. Despite its abstraction, the model produces non-linear behavior and reproduces the dynamic structure of the inflammatory response. Increasing only the initial perturbation (injury) to the system produces a state akin to SIRS. Furthermore, adjustment of the simulation to model the failed initial anti-inflammatory trials of the 1990's demonstrates the adverse outcome that was seen in those clinical trials. It is hoped that building ABCSs of sufficient sophistication may eventually provide an important bridging tool to translate basic science discoveries into clinical applications. Building these simulations will require a large collaborative effort, and it is hoped that this paper will stimulate interest in this form of analysis.

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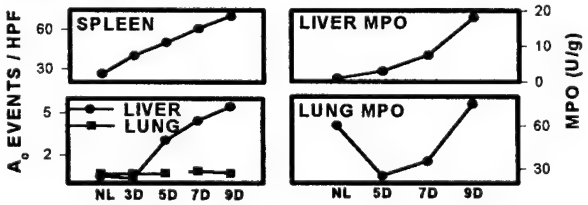
APOPTOSIS IS INDUCED IN MOUSE LIVER AND SPLEEN DURING SEPSIS. John F. Kuhn*, Stephen A. Rowe*, Glen A. Franklin*, James C. Peyton* and William G. Cheadle. VAMC and Dept. of Surgery, University of Louisville, 800 Zorn Ave. Louisville, KY 40206.

PMNs play a critical role in bacterial peritonitis and have a known circulatory lifespan of approximately 24 hours. Previous experiments have shown that circulating PMNs show little change in percent apoptosis when harvested and immediately fixed even after exposure to stimuli known to induce apoptosis. We hypothesize that organs with macrophages exposed to the blood provide an increased clearance of apoptotic PMNs after an inflammatory insult there by keeping the percentage apoptisis for circulating PMNs relatively constant. Swiss-Webster mice (n=8 per group) underwent a 23ga. cecal ligation and puncture (LD-25). Liver, lung and spleen samples were collected for in situ TUNEL staining at various time points. Tissue slices were stained with an APO-BRDU TUNEL kit with methyl green counter stain. Apoptotic (A_o) nuclei were counted under high power on an standard light microscope. Results were averaged for 25 fields and expressed as events per high power field (HPF) for each tissue sample. Lung showed no significant increase in apoptosis over the time course while liver showed significant increases at the 5, 7 and 9 day time points. A high baseline apoptosis was noted in splenic tissue and was significantly increased at 3, 5, 7 and 9 days. Liver and lung MPO values correlated with their respective apoptosis values.



PMNs are sequestered late after sepsis in liver and spleen but not in lung. PMN sequestration accounts at least in part, for the increased apoptosis in the liver and spleen late after a septic insult.

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SHOCK WAVE EXPOSURE CAUSE ENDOTHELIAL ACTIVATION AND CELL DEATH

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High-energy trauma, such as gunshots and explosions, expose the human tissues to shock waves. Several publications have demonstrated injury to the vascular endothelium associated with shock wave exposure. Therefore, we have studied the effects of shock waves on endothelial monolayers *in vitro*. Briefly, a Flyer-plate model was used to expose human umbilical cord vein endothelial cells (HUVECs) to 23.0 ± 5.2 (Mean ± SEM) MPa with or without simultaneous cavitation. Effects on cell morphology, expression of P-selectin and the distribution of actin filaments, were studied using phase contrast microscopy, computerized morphometry and immunocytochemical staining techniques. Cell necrosis/apoptosis was detected using Annexin V-propidiumiodide. HUVECs exposed to shock waves solely, did not exhibit any changes in the studied parameters, when compared to controls. In contrast, all HUVEC cultures exposed to a shock wave and cavitation (SAC) displayed areas of 6.76 ± 0.85 mm^2 with cell necrosis and central cell loss (n=14). Expression of P-selectin (n=6) as well as the disruption of actin dense peripheral bands (DPB:s) and induction of stress fibers (n=8) was demonstrated.
In conclusion, SAC caused endothelial cell activation and defined cell death. In the in vivo situation the expression of P-selectin and disruption of DPB:s may promote the adherence of platelets and leucocytes to the endothelium and the migration of leucocytes into the surrounding tissue.

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DAILY HIGH DOSE L-CARNITINE ADMINISTRATION IN INTRA ABDOMINAL SEPTIC ABSCESS INDUCES PARTIAL REVERSAL OF MUSCLE PDH INHIBITION AND DECREASES PLASMA GLUCOSE. M. Manco*, J. H. Siegel, N. Espina, C. Idler*, J. R. Lussier. UMDNJ-New Jersey Medical School, Newark, NJ 07103.

The effect of high dose L-Carnitine (300 mg/kg day) on glucose metabolism was studied after the creation of a sterile (SI) or septic (SA) intraperitoneal (IP) abscess in the rat. Five groups of animals were studied: Control (C), SI+saline (SI), SI +carnitine (SI+CARN), SA+saline (SA), and SA+carnitine (SA+CARN). The septic abscess was created by inoculating a sterile IP pellet with *E. coli* 10^2 and *B. fragilis* 10^8. SA induces increased proteolysis, gluconeogenesis, and ureagenesis due to post receptor inhibition of muscle pyruvate dehydrogenase activity (PDH_a) with an increase in plasma glucose (GLUC) and

branched chain amino acids (BCAA). L-carnitine (CARN) reduces both SI and SA proteolysis, urinary glucose and urea excretion. It was studied for its effect on muscle and liver PDH_a, GLUC and BCAA. Five days after abscess formation and saline or CARN injections (SC), frozen samples of posterior thigh muscle and liver were analyzed for percent PDH_a. SI animals showed no alteration in % muscle PDH_a from Control (C 28.3% vs SI 30.1%, NS) but liver PDH_a increased from control (C 11.3% to SI 27.2%, SA 21,2% p<.001). CARN administration did not effect SI muscle PDH_a, but, in SA, muscle PDH_a was reduced from control (C 28.3% and SI 30.1% to SA 11.8%, p<.0001,) but there was no SA change from C or SI in liver PDH_a. The addition of CARN in SA partially reversed the muscle PDH_a inhibition (11.8% to 14.3%, p<.0001) but did not effect liver PDHa (18.4%, NS). Plasma GLUC was inverse to muscle PDH_a, increasing from 8.8mM in C to 10.3mM in SI, but was markedly increased in SA 13.9mM, p<.001. CARN in SA reduced GLUC toward the SI level (12.0mM, p<.001). Of BCAA, CARN reduced only valine (SA 96.7*u*M vs SA+CARN, 91.2*u*M, p<.05). CARN also reduced daily 3-methylhistidine and urea excretion in SA, we suggest that CARN may partially reverse PDH_a inhibition by enhancing non glucose oxidative fuel use, thus reducing substrate end-product inhibition of PDH_a.

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HIGH ENERGY PHOSPHATES AND RECOVERY OF CARDIAC PERFORMANCE IN ENDOTOXIN SHOCK. L.Vona-Davis, P. Wearden*, D. Murray*[1] and R. Hill*, Dept. of Surgery, West Virginia University and [1]NIOSH, Morgantown, WV 26506.

Early reports have attributed cardiac failure during acute models of endotoxin shock to a lack of high-energy phosphates. In the present study we investigated the relationship between myocardial performance and energy metabolism in the rat at 4 and 16 h after a bolus injection of *E. coli* endotoxin (5mg/kg). Cardiac function was evaluated using the isolated perfused working heart preparation. To assess the adequacy of energy metabolism, freeze-clamped hearts were obtained from a separate group of animals to study the concentrations of endogenous ATP, phosphocreatine (PCr), inorganic phosphate (P_i) and intracellular pH by ^{31}P-cryo-magnetic resonance spectroscopy. At 4 h after exposure to endotoxin, cardiac output and stroke volume were reduced by 11% and 12%, respectively when compared with controls. Left ventricular function ($+dP/dt$) was also significantly reduced by 9% ($P < 0.05$). By 16 h, however, all cardiac parameters had normalized. Intact hearts taken for high-energy phosphate analysis at both times revealed minor alterations in PCr, ATP or P_i content. ATP and PCr concentrations at 4 h after endotoxin were similar to controls while P_i increased by more than 50% ($P < 0.05$). By 16 h, ATP levels decreased 31%, but this change was not significant. Intracellular pH of the heart was unaltered by duration of exposure to this level of endotoxin. Ratios of PCr/ P_i, an index of energy stores or work capacity, declined by 50% at 4 h but recovered completely by 16 h. This correlates with cardiac performance and suggests that the ability of the heart to perform mechanical work was impaired early in endotoxin shock. Elevation of PCr/ATP ratios ($P < 0.01$) suggests that the aerobic capacity of the heart was augmented to meet the equilibrium between energy stores and ATP concentrations, thus enabling it to recover from contractile dysfunction.

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THROMBIN-INDUCED MICRO- AND MACRO-VASCULAR ENDOTHELIAL PERMEABILITY IS RELATED TO EARLY CALCIUM FLUX. SL Duffy* and JT Murphy. UT Southwestern Med. Center, Dallas TX 75235-9158

Although all endothelial cells form a selective barrier for the passage of solutes to and from the vasculature, functional differences exist dependent on the vascular bed of cell origin. This study examines endothelial permeability and intracellular calcium flux responsible for thrombin-induced monolayer barrier leak in human microvascular lung and macrovascular umbilical vein endothelial cells. HMVEC-L and HUVEC were obtained by trypsinization of lung and umbilical veins, respectively. LDL uptake, Factor VIII staining and cobblestone morphology confirmed cell type. On 0.4μM pore polyester membranes confluent monolayer permeability was assessed as diffusion of biotinylated bovine serum albumin across monolayers over time. Select cells were pre-treated for 30 minutes with Fura-2/AM (4μM) prior to activation with 10nM human thrombin. To measure intracellular calcium flux, cells were loaded with 2μM Fura-2/AM for 45 minutes, stimulated with thrombin and spectrophotometrically analyzed at 510nm (excitation wavelengths 340 and 380nm). Thrombin-stimulated HUVEC calcium concentrations were increased 100x within 55 seconds, then decreased to near-basal levels by 8 minutes, while lung EC calcium increased 15x in 120 seconds and resolved by 14 minutes. By 30 minutes, permeability of HUVEC monolayers had increased 9-fold and HMVEC-L by 50x over controls. Pre-treatment of HUVEC and HMVEC-L with Fura-2/AM diminished thrombin-mediated permeability to less than 67% and 40% of maximal respectively. While thrombin-induced permeability appears to be mediated by a calcium mechanism in microvascular and macrovascular endothelial cells, the magnitude and duration of calcium flux, as well as the degree of monolayer permeability, was unique to the individual endothelial cell types.

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DILTIAZEM REDUCES LEUKOCYTE ADHESION AFTER HEMORRHAGIC SHOCK IN THE RAT. H. Jakob*, W. Kuntz*, I. Marzi, S. Rose. Univ. Saarland Trauma Surgery, 66421 Homburg, Germany

Microcirculatory failure and increased leukocyte adhesion to the hepatic endothelium following hemorrhagic shock induce hepatic dysfunction. Previous own studies showed that Ca^{2+} channel blocker diltiazem (DZ) decreased activation, Ca^{2+} concentration and tissue invasion of leukocytes after shock. Aim of the study was to evaluate effects of DZ on hepatic sinusoidal perfusion and leukocyte adhesion after hemorrhagic shock. *Methods.* Anesthetized, male Sprague-Dawley rats (~250g, $n \geq 5$) were hemorrhaged to 40 mmHg for 60 min and resuscitated with 60% of citrated shed blood and Ringer's lactate. DZ was infused at a dose of 0.8

mg/kg during the first hour of resuscitation. Microcirculation in the liver sinusoids [white blood cell (WBC) velocity, WBC endothelium interactions] was examined by intravital fluorescence microscopy 24 hrs after shock. Statistics: One-way ANOVA, mean ± SEM. *Results.* Leukocyte adhesion was significantly reduced in DZ-treated shock rats (1877.8 ± 108 cells/mm^2) as compared to untreated shock rats (4041.4 ± 74 cells/mm^2, $p < .05$). DZ had no effect on diameters or WBC velocity of sinusoids. *In conclusion.* The fact that diltiazem modulated shock-induced leukocyte-endothelial adhesion without affecting vessel diameters and leukocyte flow suggested a potential role of leukocyte and/or endothelial Ca^{2+} regulation in post-ischemic adhesion mechanisms.

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Intermittent hypoxia induces vasoregulatory gene expression N Sonin, Y Yokoyama, JX Zhang and MG Clemens. Dept of Biology, Univ. North Carolina at Charlotte, Charlotte NC

Although the effects of reoxygenation after sustained hypoxia are well recognized, the response to brief intermittent hypoxia (IH) is not well understood. In this study we used a rat model of acute IH to determine its effect on systemic circulation and expression of major vasoregulator genes in various organs. Methods. Anesthetized rats were mechanically ventilated; IH was induced by stopping the ventilator for 60 seconds each five minutes. Fifteen cycles were applied. Blood pressure and heart rate were continuously monitored. Five hours after IH samples of organs were harvested for RT-PCR analysis of RNA. Results. BP was decreased to 27-30 mm Hg at each hypoxic episode and recovered to baseline at normoxia.. Changes in vasoregulatory genes mRNA level are summarized in the table as fold over control.

organ/mRNA	ET-1	ET-A	ET-B	iNOS
heart	1.2	NA	1.0	1.7
lung	0.9	0.6	0.9	1.5
intestine	2.7	1.5	1.4	3.2
liver	1.2	1.0	1.2	1.7
kidney	1.6	NA	1.0	1.4
spleen	1.0	NA	1.8	1.4

Conclusion. These data show that even brief periods of IH have substantial effects on expression of major vasoregulator genes, especially endothelins and NO. These changes can result in disturbed microcirculation and tissue damage. The sensitivity of the intestine indicates suggests an important role in in whole body O_2 sensing during brief periods of intermittent hypoxia. Both ET-1 and NO may be significant contributors to altered vasoreactivity following intermittent hypoxia.

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HYPOXIA (H) CAUSES INCREASED BLOOD COAGULABILITY WHEN DETERMINED UNDER HIGH SHEAR (HS) BUT NOT UNDER LOW SHEAR (LS) CONDITIONS. CR Spillert, UMDNJ-NJ Medical School, Newark NJ 07103

Hypoxia in association with disseminated intravascular coagulation (DIC) is observed in trauma patients with organ failure. Whether hypoxia can generate a state of activated blood coagulation, a precursor to thrombosis, was the purpose of this study. Human citrated whole blood (CWB) was placed in plastic petri dishes at atmospheric pressure (AP) or made hypoxic by placing under vacuum for two hours. The CWB was recalcified (to neutralize the citrate and initiate the clotting process) in a Sonoclot Coagulation Analyzer II. Recalcification time (RT) measured under LS employed the standard hollow probe which vibrates axially in the clotting chamber and detects increased viscosity as fibrin forms. HS was induced by sealing the end of the probe in contact with blood with wax (i.e. 50 times the surface area). The RT values (n=8) ± SD (sec) are as follows: AP+LS 301±94; H+LS 310±99; AP+HS 320±114; H+HS 181±24. The RT of the hypoxic aliquot evaluated at high shear was significantly reduced ($P<0.01$) when compared to the other values. Hypoxic blood when measured under LS did not alter RT. However, RT measured under HS detected a hypercoagulable state. This state could, in part, reflect changes in cell membranes (receptor sites), generation of procoagulants or inhibition of the fibrinolytic system. Whether this assay could be of diagnostic utility in trauma patients warrants further study.

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Functional role of endothelin receptor subtype distribution in endotoxemia Y Yokoyama, R Baveja, N Sonin, K Nakanishi, JX Zhang and MG Clemens Dept of Biology, Univ of North Carolina at Charlotte, NC 28223

We previously showed that endothelin receptor subtypes are heterogeneously distributed in the liver microcirculation (ET_B receptors dominating in the terminal hepatic venules at the outflow from the sinusoids). Endotoxemia also specifically upregulates ET_B receptors. Therefore, this study was performed to determine the functional role of heterogeneous distribution of ET receptors. Distribution of ET_A and ET_B receptors was determined by in situ binding and autoradiography. ET_A receptors were restricted to the portal triads while ET_B receptors were found in both inflow and outflow. Functional significance was determined by measurement of weight changes in isolated perfused liver to determine rate of change of vascular and extracellular volume. Controls showed only transient decrease in weight consistent with decreased vascular compliance. In contrast, in endotoxic rats (ET_B upregulated), the initial decrease was followed by a

dramatic increase (> 50% of total liver weight) indicating outflow obstruction. Pretreatment with L-NAME abrogated this effect indicating a contribution from ET_B dependent NO production. Intravital microscopic observation showed disruption of normal flow patterns with frequent retrograde sinusoidal flow with ET_B receptor activation. These results demonstrate that ET_B receptors regulate both inflow and outflow resistance in the liver microcirculation. Moreover, upregulation of ET_B receptors in endotoxemia can contribute to disruption of flow patterns, outflow block and highly heterogeneous distribution of sinusoidal flow. Supported by DK 38201

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PROGRESSIVE DECREASE IN CONSTRICTOR REACTIVITY OF THE NON-ABSORBING INTESTINE DURING CHRONIC SEPSIS

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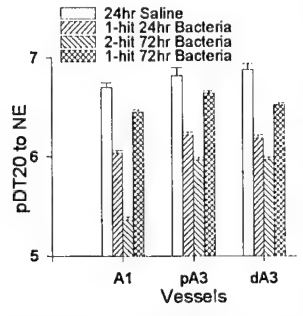
Chronic sepsis leads to an impaired intestinal microcirculation, which might reflect altered microvascular control. We hypothesized that intestinal microvascular sensitivity to norepinephrine (NE) is decreased during chronic sepsis. Chronic sepsis was induced by inoculation *(E.coli and B.frag)* of implanted subcutaneous sponges in rats. Septic rats were studied either at 24 hr or 72 hr after a single inoculation (1-hit) of bacteria. Other rats received two inoculations (2-hit) of bacteria and were studied at 24 hr after the 2nd inoculation. NE (0.01 – 1.0 μM) responses in the non-absorbing terminal ileal arterioles (inflow A1, proximal-p and distal-d premucosal A3) were measured by video-microscopy. NE threshold sensitivity (pDT20= -log of 20% response dose) was analyzed by ANOVA-SNK. pDT20 was significantly decreased in A1, pA3 and dA3 of 1-hit 24hr septic rats; and was further decreased in all three vessels of 2-hit 72hr septic rats (Fig). In contrast, the pDT20 of all three vessels significantly returned toward normal values after 72 hr in rats that had only 1 bacteria inoculation (Fig). We conclude that an initial bacterial challenge decreases vasoconstrictor control of the intestinal microcirculation, and that subsequent repeated bacterial challenge exacerbates this defect in vasoconstrictor control in the non-absorbing intestine. (Funded: VA Merit, Dept. of Defense, CAMR)



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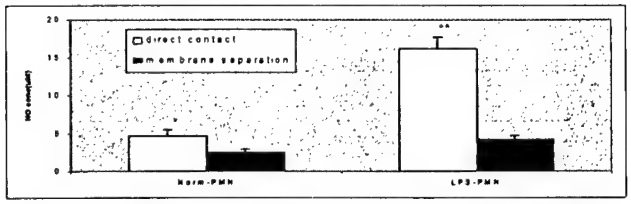
NITRIC OXIDE PRODUCTION IN KUPFFER CELL-NEUTROPHIL CO-CULTURE IS DEPENDENT ON CELL-CELL INTERACTION.

Ketan Sheth*, Andrew Duffy*, Mike Potter*, Paul Bankey. Univ. of Massachusetts Medical School, Worcester, MA 01655.

Nitric Oxide(NO) plays a variety of roles in the inflammatory response to shock. Sequestration of neutrophils (PMN) has been implicated in hepatic dysfunction following shock. Previously we reported that activated PMN were a potent stimulus for NO production in Kupffer cell-PMN co-cultures. We hypothesize that PMN-Kupffer cell adhesion is required for NO induction. To test this, Male Wistar rats (250g) were injected with intraperitoneal LPS(E.coli 0111:b4, 4mg/kg in 10cc) or intraperitoneal normal saline(10cc). After 18 Hs neutrophils(>95%PMN) from LPS treated(LPS-PMN) or saline treated (Norm-PMN) animals were isolated by Ficoll Gradient centrifugation and RBC sedimentation. KC were harvested from healthy male Wistar rats(>80% purity via phagocytosis assay). A 0.4 micron diffusable polyethylene terephthalate track-etched membrane(PET) was then used to separate the two cell types. Supernatants were collected at 18 Hs from cultures with and without the PET membrane and analyzed via the Greiss reaction.



The nitrite production in the culture systems that had direct contact between KC-PMNs was significantly increased compared to the co-cultures that were separated by the PET membrane(*$p<0.05$ by ANOVA). Placing a membrane between the cells abrogates the dramatic rise in nitrite production. These data suggest that direct KC-PMN contact rather than release of soluble signals is necessary for induction of nitric oxide. This potentially offers another avenue of regulation of nitric oxide production in gram-negative infection.

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SYSTEMIC EFFECTS OF FLAGELLIN AND LIPOPOLYSACHARRIDE, TWO VIRULENCE FACTORS OF GRAM-NEGATIVE BACTERIA

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Flagellin (Flg), the monomeric subunit of bacterial flagella, may be involved in the virulence of Gram negative (G^-) bacteria. In this work, we compared the systemic response triggered in conscious male Balb/C mice by the intravenous injection (tail vein) of either LPS (*E. Coli*, 5 mg/kg; n=14) or recombinant Flg (*Salmonella muenchen*, 5 mg/kg; n=13). At 4 and 8 h, samples of lung and liver were harvested for the determination of neutrophil infiltration (myeloperoxidase, MPO), lipid peroxidation (malondialdehyde, MDA) and glutathione (GSH) levels. Blood samples were assayed for the levels of transaminases (ASAT+ALAT) and nitrate ($NO3^-$, Griess reaction). The results are given in the table.

Variable	LPS 4h	FLG 4h	LPS 8h	FLG 8h
Lung MDA	0.5±0.1	0.8±0.2	0.5±0.1	0.8±0.1*
Liver MDA	1.6±0.2	2.4±0.3*	2.1±0.3	3.7±0.3*
Lung MPO	164±13	259±43*	168±11	196±25
Liver MPO	25±8	23±11	19±6	19±6
Liver GSH	157±5	129±12*	120±22	72±8*
Transam.	154±17	277±68*	156±28	159±13
$NO3^-$	272±20	210±14*	444±21	376±26

Means±s.e.m. *p<0.05 FLG vs LPS at same time-point. MDA:nmol/mg. MPO:mU/mg protein. GSH:µg/100 mg. Trans:UI/L. $NO3^-$:µmol/L
Flg induced oxidant-mediated damage in the lung and liver, and recruited neutrophils in the lung more potently than LPS. In contrast, although both LPS and Flg induced NO production, this effect was more pronounced with LPS. These data indicate that Flg may represent a novel mediator of G⁻bacteria-mediated inflammation.

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SYSTEMIC ADMINISTRATION OF FLAGELLIN INDUCES HYPOTENSION AND *EX VIVO* VASCULAR DYSFUNCTION IN MICE

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Vascular dysfunction in gram negative sepsis is mediated by the production and releases of pro-inflammatory mediators that upregulate expression of inducible nitric oxide synthase (iNOS) in the vasculature. Bacterial lipopolysaccharide (LPS) is a prototypical bacterial component which mimics many consequences of shock and sepsis. However LPS may not be the sole pro-inflammatory stimulus released from bacteria, as polysaccharides, lipoteichoic acid, bacterial DNA and other factors have been identified as causative factors recently. Our group has completed a series of experiments which indicate that flagellin, a protein component of flagellated bacteria, induces iNOS expression and pro-inflammatory response in vivo. Here we studied the hemodynamic and vascular effects of administration of flagellin in Balb/c and C3H/HeJ (LPS-resistant) mice. Mice received recombinant S. muenchen flagellin (10 mg/Kg) or vehicle (PBS) 200 µl iv. The mean arterial pressure remained at baseline levels (100mmHg) for approx. 2 hours, followed by severe hypotension (50 mmHg), cyanosis and death between 2-4 hours. Vehicle–treated controls were normotensive during the 4-hour period of observation. Ex vivo vascular reactivity was studied in aortic ring preparations. Isometric force was measured and concentration-response curves to phenylephrine (10^{-10} to $3x10^{-5}$ M) and acetylcholine (10^{-9} to $3x10^{-5}$M) were constructed. A significant reduction in vascular contractility and endothelium-dependent relaxation developed in animals challenged with flagellin. Flagellin produced similar in vivo hemodynamic and ex vivo vascular effects in wild-type and LPS-resistant C3H/HeJ mice. Systemic NO production (circulating nitrate) was increased in the animals in response to flagellin. Thus, flagellin may be a novel mediator of vascular dysfunction in certain forms of bacteria induced shock.

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iNOS ACTIVITY AND CARDIAC DYSFUNCTION IN AN ENDOTOXIN MOUSE MODEL.

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The pathophysiologic role of nitric oxide in septic shock induced cardiac dysfunction continues to be debated. The purpose of this study was to examine the role of iNOS in acute endotoxin induced cardiac dysfunction using an iNOS knockout mouse model.

We evaluated iNOS protein levels, NOS activity and cardiac function in C57/BL6 wild type and iNOS knockout mice following intraperitoneal injections of 200 µg *E.coli* LPS. Myocardial iNOS protein and serum nitrite levels were determined at 4, 8, 12 and 18 hours post LPS injection. Langendorff preparations were used to evaluate *in vitro* cardiac function 18 hours after LPS challenge.

Myocardial iNOS protein, and serum nitrite levels were increased in the wild type mice following LPS injection. There was no demonstrable iNOS protein or nitrite in the iNOS knockout mice. In both wild type (LVP:68+/-11, +dP/dt:1526+/-310, -dP/dt:1000+/-265) and iNOS knockout (LVP:63 +/115, +dP/dt:1625+/-388, -dP/dt:1145+/-297) mice, there was a decrease in left ventricular function following LPS challenge as compared to control (LVP:96 +/-5, +dP/dt:2217+/-147, -dP/dt:1836+/-180) animals. However the iNOS knockout mice retained β-adrenergic sensitivity while wild type mice had a decreased response to isoproterenol.

We concluded that while nitric oxide is not directly responsible for endotoxin induced myocardial dysfunction, it behaves as a negative inotrope by triggering a decrease in cardiac contractility to β-adrenergic stimulation.

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TRANSGENIC MODEL OF I-κB OVEREXPRESSION: TO EXAMINE THE ROLE OF NF-κB IN POSTBURN MYOCARDIAL CONTRACTILE DYSFUNCTION.

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Our previous studies have shown that burn trauma activates cardiac NF-κB, promotes TNF-α synthesis and contractile dysfunction. Recently, transgenic mice which overexpress I-κB specifically in the heart have been developed. The I-κB overexpressors are driven by the α-myosin heavy chain promoter; therefore, NF-κB translocation is inhibited only in cardiac myocytes. This study examined the effects of I-κB overexpression on cardiac performance after burn trauma. I-κB overexpressor mice (I-κB/OE) and appropriate wild types were given a 3° scald burn over 40% TBSA and fluid resuscitated (IP). Wild type shams and I-κB/OE shams were included for controls; 24 hrs postburn, hearts (N=7-9/group) were perfused (Langendorff). There were no significant differences in cardiac function in the wild type sham compared to I-κB/OE shams. Burn trauma in wild type mice impaired cardiac performance as indicated by lower developed LVP (mmHg) and ±dP/dt max (mmHg/sec). In contrast, postburn cardiac performance was significantly improved in I-κB/OE burns compared to that observed in the wild type burns. We conclude that activation of the NF-κB pathway plays a significant role in postburn cardiac contractile dysfunction.

	Wild Sham Type	I-κB/OE Sham	Wild Type Burn	I-κB /OE Burn
LVP (mmHg)	92.±3	96±4	68±4*	81±5
+dP/dt max (mmHg/sec)	2161±39	2319±72	1635±124*	1921±111
-dP/dt max (mmHg/sec)	1757±75	1854±87	834±86*	1515±119

**** indicates significant difference, p<0.05***

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MYOCARDIAL ISCHEMIC PRECONDITIONING IS DEPENDENT ON POLY (ADP-RIBOSE) SYNTHETASE

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Cell necrosis triggered by the excessive activation of the nuclear enzyme poly (ADP-ribose) synthetase (PARS) in response to oxidant-mediated DNA injury has been shown to play an important role in the pathogenesis of various forms of reperfusion injury and shock. The aim of the present study was to investigate the role of PARS in myocardial ischemic preconditioning (IPC), by comparing the response to IPC, followed by myocardial ischemia/reperfusion, in wild-type and PARS deficient animals. Mice with genetic disruption of PARS and littermate wild-type animals underwent 30 min occlusion of LAD and up to 24 hours reperfusion. For ischemic preconditioning, four cycles of 5 min occlusion and 5 min reperfusion were applied **prior to** occlusion. PARS deficient mice were protected from reperfusion injury and showed an attenuated inflammatory mediator production and reduced neutrophil infiltration. IPC also induced a significant protection against myocardial reperfusion injury in the $PARS^{+/+}$ mice, which was also associated with attenuated inflammatory mediator production and reduced neutrophil infiltration. This protection was associated with partially preserved myocardial NAD levels, indicating that PARS activation may be attenuated by IPC. Surprisingly, the protective effect of IPC not only disappeared in $PARS^{-/-}$ mice, but even a marked enhancement of the myocardial infarction was observed. Taken together, the current results suggest that the mode of IPC's action is related, at least in part, to an inhibition of PARS. We speculate that this process may occur either by self-auto-ribosylation (i.e. inactivation) of PARS during the period of IPC, and/or via the release of purines during ischemia which inhibit PARS activation during reperfusion.

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SEPSIS INDUCED ALTERATION IN T-CELL SIGNALING IN NEONATAL RATS.

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Sepsis-induced suppression in T-cell proliferation follows deranged Ca^{+2} signaling in adult rats. In our preliminary studies, we observed suppression in T-cell proliferation in septic neonatal rats as well. In this study, we assessed T-cell cytosolic Ca^{+2} concentration, $[Ca^{+2}]_i$, as its elevation plays an important role in T-cell proliferation. Also, we investigated the role of PGE2 in sepsis-related changes in T-cell $[Ca^{+2}]_i$ by pre-treating animals with COX-I inhibitor (Resveratrol) and COX-II inhibitor (NS-398). Sepsis was induced in 15 day old rat pups by intra-peritoneal implantation of fecal pellets containing E. Coli and B. Fragilis. The sham group consisted of pups implanted with sterile fecal pellets. Septic, sham and unoperated control pups were sacrificed 24 hrs after implantation and their spleens removed. The spleens were processed for single cell suspensions, and T-cells were isolated using nylon wool coloumns. Fura-2 flurophotometry was employed for the measurement of $[Ca^{+2}]_i$ (in nM units) in T-cells stimulated with ConcanavalinA (ConA).

	Control	Sham	Sepsis
Untreated	162 ± 7.41	141 ± 9.96	97 ± 6.26
Resveratrol	-	150 ± 10.3	98 ± 4.95
NS-398	-	157 ± 9.14	171 ± 7.69

Our results show that ConA-mediated T-cell $[Ca^{+2}]_i$ response is significantly suppressed in septic neonatal rats. Pre-treatment of pups with COX-2, but not COX-1 inhibitor prevented the decrease in the $[Ca^{+2}]_i$ response. These findings suggest that PGE2 could induce the attenuation in T-cell Ca^{+2} signaling during sepsis in neonatal rats. (Support from Department of Pediatrics/Neonatology, Loyola University Medical Center, and from NIH GM 53235 and GM 56865).

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***IN VIVO* DETERMINATION OF THE EFFECT OF PEEP ON HYPOXIC PULMONARY VASOCONSTRICTION**

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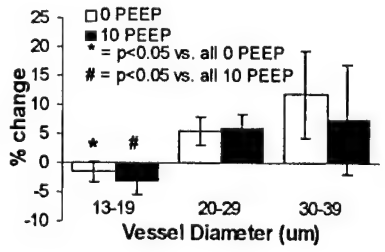
Introduction: Although hypoxic pulmonary vasoconstriction has been investigated in a number of clinical conditions (lung inflation, atalectasis, pulmonary hypertension) the effect of positive end expiratory pressure (PEEP) has yet to be defined. This study tested the hypothesis that PEEP would decrease the HPV response in pulmonary microvessels. **Methods:** Using an *in vivo* porcine model, sub-pleural vessels were observed by videomicroscopy. Hemodynamic data and vessel diameter were recorded at normoxia (FiO2=1.0) and hypoxia (FiO2=0.15) at 0 PEEP and then at 10 PEEP. Each vessel served as its own control. **Results:** We observed that HPV varied directly with vessel diameter (see graph). HPV was greatest in larger vessels (30-39μm--11.8%±7.6) and less in smaller vessels (20-29μm--5.5%±2.3). HPV was absent in vessels 13-19μm (-1.5%±1.8). The application of PEEP caused no significant change in any of these vessel diameters. **Conclusion:** PEEP has no measurable effect on the HPV response in pulmonary microvessels.



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REGIONAL ALTERATIONS OF RENAL BLOOD FLOW FOLLOWING INTESTINAL ISCHEMIA/REPERFUSION (II/R). K.Bradley*,A.R.Seelig*,L.Bartula*,R.Milner*, M.Ruggieri* and S. Myers, Temple Univ., Phila., PA 19140.

This study examines the hypothesis that intestinal ischemia/ reperfusion (II/R) produces alterations in regional blood flow within the kidney. A secondary aim of this study is to determine if use of implantable laser doppler flow probes are more sensitive to changes in renal blood flow (RBF) than renal artery ultrasonic flow probes. Anesthetized male Sprague Dawley rats (325-350g) had total RBF (tRBF, reported as mls/min) measured with a Transonic renal flow probe and cortical (cRBF) and medullary (mRBF) RBF measured with Perimed Laser Doppler single fibre probes inserted into the kidney at 2 and 4mm, respectively (reported as perfusion units). Blood pressure (BP) was monitored and reported as mm Hg. Animals were subjected to sham (SM, 180 min.) or intestinal ischemia/ reperfusion (II/R, 120/60 min) via superior mesenteric artery occlusion . Readings were recorded every 10 minutes throughout the II/R or SM procedures and reported for the 120-160 min. reperfusion period. Data was reported as mean % basal±SEM and analyzed by two-tailed t test (N≥4,*-p<0.01 compared to appropriate SM).

	120'	130'	140'	150'	160'
BP-SM	74±7	77±5	75±5	68±5	77±4
BP-II/R	80±5	59±5	59±3	57±2	52±3
tRBF-SM	48±9	47±9	42±9	38±8	36±6
tRBF-II/R	57±2	29±2	24±2	18±3	15±2.5*
cRBF-SM	83±12	89±15	84±15	105±3	94±17
cRBF-II/R	90±12	65±13	66±15	57±18	59±16
mRBF-SM	122±16	125±10	123±11	128±9	126±9
mRBF-II/R	78±11*	60±7*	59±7*	59±6*	53±15*

These data show that II/R profoundly alters distribution of intra-renal blood flow with the most significant decreases found in the medulla. These data also show that use of implantable laser doppler probes identify the II/R induced decrease in renal blood flow earlier than use of ultrasonic renal artery flow probes that measure total renal blood flow.

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Protective effect of *n*-acetylcysteine, a free radical scavenger, administration on gentamicin-induced acute renal failure in rats.

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We investigated the effects of *n*-acetylcysteine (NAC) in gentamicin-induced acute renal failure. After 8 days, the gentamicin (100 mg/kg/day s. c), group developed a marked renal failure, charactepized by a significantly decreased creatinine clearance and increased blood creatinine levels, and daily urine volume when compared to controls. Kidney myeloperoxidase (MPO) activity and lipid peroxidation was significantly increased in gentamicin-treated rats. Immunohistochemical examination demonstrated a marked increase in the immunoreactivity to nitrotyrosine and PARP in the kidney of gentamicin-shocked rats. Renal histological examination confirmed tubular necrosis. Pretreatment of gentamicin-shocked rats with *n*-acetylcysteine (10 mg/kg, intraperitoneally, daily) prevented the development of renal failure.

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LPS-induced, Imbalanced Expression of Hepatic vascular Stress Genes in Cirrhosis: Mechanism of Increased Susceptibility to Endotoxemia. R Baveja, Y Yokoyama, X Bian, N Sonin, MG Clemens and JX Zhang. Dept. of Biology, Univ. of NC Charlotte, NC 28223.

Cirrhotic patients are more susceptible to endotoxemia in giving rise to hepatic injury. The mechanism is not completely understood although endotoxemia has been shown to cause hepatic microcirculatory failure, which has been blamed for subsequent liver damage. An important element in the hepatic microcirculatory dysfunction during endotoxemia is an imbalance of locally released vasoconstrictors and vasodilators. We studied the expression of the genes responsible for vascular regulation in the cirrhotic liver superimposed by endotoxemia. Cirrhosis was induced by bile duct ligation (BDL) in male SD rats for 21 days. Endotoxemia was induced by injecting LPS (i.p., 1 mg/kg). Plasma and liver samples were taken 6 hr later for ALT assays and analysis of expression of endothelin (ET-1), its receptors ET_A and ET_B, inducible nitric oxide synthase (iNOS) and hemeoxygenase (HO-1) by a semi-quantitative RT-PCR, respectively. ALT increased 5.5 fold in the BDL animals as compared to sham and was exacerbated (9.3 fold increase over sham) with endotoxemia. Both LPS and BDL alone significantly increased ET-1 mRNA (1.6 and 1.7 fold increase over sham, respectively), which, however, was further induced with endotoxemia following BDL (2.2 fold increase over sham). ET_A mRNA decreased in BDL animals that showed no further changes with endotoxemia. LPS increased ET_B mRNA in sham but no significant change was observed in BDL with endotoxemia. Among the vasodilator forces, iNOS induction in BDL animals with endotoxemia was minimal as compared to the marked increase in sham treated with LPS (4.3 vs. 56.5 fold increase over sham, respectively). No significant induction of HO-1 was found in BDL animals treated with LPS while a markedly increased expression was observed in sham with endotoxemia. Taken collectively, significantly greater induction of the constrictive forces (i.e. ET-1) over the dilatory forces (i.e. iNOS and HO-1) was observed in liver cirrhosis superimposed with endotoxemia, suggesting a compromised ability of the cirrhotic liver in upregulating sufficient dilatory forces to counterbalance the constrictive effect of ET-1 upon a secondary endotoxemia, which may at least partly explain the increased susceptibility.

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ROLE OF NITRIC OXIDE IN HEMORRHAGIC SHOCK-INDUCED HEPATIC HEME OXYGENASE-1 EXPRESSION IN THE RAT A. Hoetzel*, D.Vagts*, T. Loop*, M. Humar*, H. Pahl*, K. Geiger*, B. Pannen. Department of Anesthesiology and Critical Care Medicine, University of Freiburg, Hugstetter Str.55, D-79106 Freiburg, Germany

Recent evidence suggests that the hepatic expression of heme oxygenase-1 (HO-1) may act to preserve

hepatocellular integrity after hemorrhagic shock and resuscitation (HR). However, the factors that regulate HO-1 gene expression under these conditions remain to be identified. Since nitric oxide (NO) has been shown to modulate HO-1 expression in cultured cells *in vitro*, we determined its role in the regulation of HO-1 expression after HR in the rat liver *in vivo*. HO-1 mRNA and protein were highly induced after HR as compared to control. In contrast, if the NO-donor molsidomine (MOL; 3 mg/kg BW) was administered before resuscitation, no induction of HO-1 mRNA or protein could be observed in response to HR. Moreover, HO-1 mRNA accumulation was much more pronounced in the presence of the NO synthase inhibitor L-NAME (1 mg/kg BW) as compared to MOL. Electrophoretic mobility shift assays revealed that the HO-1 induction after HR was associated with an increased DNA binding activity of the transcription factor activator protein-1 (AP-1) that was completely abolished by MOL. In contrast, DNA binding activity of the nuclear transcription factor-κB (NF-κB), another factor that binds to a cis-acting element within the HO-1 promotor, did not differ among groups. In conclusion, our results suggest that NO may attenuate hepatic HO-1 gene expression after HR. This NO-mediated suppression of HO-1 induction in response to HR may be due to an inhibition of the DNA binding activity of AP-1. These findings could have important implications for the development of new strategies aimed at limiting hepatocellular injury after HR.

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INTERLEUKIN (IL)-6 KNOCKOUT ATTENUATES EARLY SEPSIS-ASSOCIATED HEPATIC GENE DOWNREGULATION BUT INCREASES HEPATIC NECROSIS AND DEATH. PK Kim*, NR Raj*, CA Haaxma*, and CS Deutschman, **University of Pennsylvania, Philadelphia, PA 19104**

Background: Murine sepsis induced by cecal ligation and puncture (CLP) causes jaundice and intrahepatic cholestasis and downregulates transcription of hepatocyte transporters sodium taurocholate cotransporter (Ntcp) and multidrug resistance-associated protein (Mrp2). These changes are likely mediated by IL-6. However, IL-6 also stimulates hepatic regeneration after injury. Thus the role of IL-6 in mediating hepatic injury in sepsis is unclear. **Hypothesis:** Absence of IL-6 attenuates downregulation of Ntcp and Mrp2 transcription in sepsis but is detrimental to ultimate hepatic recovery and survival. **Methods:** Studies were performed in accordance with NIH guidelines. Under general anesthesia, C57/BL6 IL-6 -/- and wild type (+/+) mice underwent single puncture CLP and were sacrificed after 0, 3, 6, 16 or 24 hrs. Fixed liver tissue was stained with hematoxylin and eosin (H&E), and hepatic nuclei were isolated for transcription elongation analysis using cDNA targets for Ntcp, Mrp2 and constitutively-expressed β2-macroglobulin. Autoradiography and densitometry were performed. H&E sections were examined for necrosis. A separate group of IL-6 +/+ and IL-6 -/- mice underwent CLP to compare survival at 0, 24, 48 and 72 hrs. **Results:** In IL-6 +/+ mice, Ntcp and Mrp2 transcription decreased to 30% of baseline by 6 hrs after CLP and returned to baseline by 24 hrs. In IL-6 -/- mice, Ntcp and Mrp2 transcription was unchanged from baseline for 24 hrs after CLP. H&E sections revealed minimal injury in IL-6 +/+ mice but significant necrosis in IL-6 -/- mice, most pronounced at 24 hrs. In IL-6 +/+ mice, there was no mortality after CLP. In IL-6 -/- mice, mortality was 75% at 48 hrs and 83% at 72 hrs. **Conclusions:** Despite preserving transcription of key hepatocyte bile acid and bilirubin transporters, absence of IL-6 worsens hepatic necrosis during sepsis and is associated with substantial late mortality. These results suggest that IL-6 mediates early hepatic dysfunction in sepsis but is necessary for ultimate hepatic recovery and survival from sepsis.

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A DOMINANT ROLE OF P55 TNF-α RECEPTOR IN ENDOTOXEMIC MYOCARDIAL DYSFUNCTION
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Previous studies by our laboratory and others have demonstrated that in vivo antagonization of TNF-α attenuates endotoxemic myocardial dysfunction. Moreover, exogenous TNF-α induces delayed myocardial dysfunction in vivo. While these studies suggest that TNF-α contributes to endotoxemic myocardial dysfunction, further studies utilizing animals lacking the ability to produce TNF-α are important to determine the role of endogenous TNF-α in this myocardial disorder. Both p55 and p75 TNF-α receptors are expressed in the myocardium. It remains unknown which TNF-α receptor mediates the cardiodepressive effect of endogenous TNF-α in endotoxemia. The **purposes** of this study were to examine whether TNF-α gene knockout renders the myocardium resistant to endotoxemia and interrogate the roles of p55 and p75 TNF-α receptors in endotoxemic myocardial dysfunction. **Methods and results:** We performed in vivo experiments using gene-targeted knockout mice lacking TNF-α, p55 or p75 TNF-α receptor. Mutant and wild type mice were treated with saline or E. coli lipopolysaccharide (LPS, 0.5 mg/kg ip). Hearts were isolated 4 hours after treatment, and left ventricular developed pressure (LVDP) was assessed by the Langendorff technique. LVDP was comparable among the groups following treatment with saline (mean 39 to 44 mmHg). LPS treatment reduced LVDP to 12±1.1 mmHg in wild type mice while it was 24±2.0 mmHg ($P<0.01$ vs wild type) in TNF-α knockout mice. Mice lacking p55 TNF-α receptor exhibited similar cardiac resistance to LPS (27±2.6 mmHg following LPS treatment, $P<0.01$ vs wild type). However, p75 TNF-α receptor knockout had no influence on endotoxemic myocardial dysfunction (14±2.7 mmHg, $P>0.05$ vs wild type). **Conclusions:** This study demonstrated that animals lacking TNF-α or the p55 TNF-α receptor are resistant to endotoxemic myocardial dysfunction. These findings suggest that p55 TNF-α receptor signaling plays a dominant role in mediating the in vivo cardiodepressive effect of endogenous TNF-α.

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EVIDENCE FOR A ROLE OF NF-κB IN ACUTE HYPOVOLEMIC HEMORRHAGIC SHOCK IN RATS.
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We investigated the role of NF-κB in acute hemorrhagic (HEM) shock. HEM shock was induced in male anesthestized rats by

intermittently withdrawing blood from an iliac catheter over a period of 20 min (bleeding period) until mean arterial blood pressure (MAP) fell and stabilized within the range of 20-30 mmHg. Electrophoretic mobility shift assay showed that liver NF-κB binding activity increased in the nucleus after 5 min of hemorrhage and remained elevated for the entire bleeding period. Western blot analysis suggested that the levels of inhibitory IκBα protein in the cytoplasm became decreased at 10 min of bleeding but were gradually restored following 20 min of bleeding. Vehicle treated rats subjected to HEM shock died within 30 min after the end of bleeding period, exhibited hypothension (MAP=20-30 mmHg), had increased levels of TNF-α mRNA in the liver (end of bleeding) and high TNF-α serum levels (800 ± 10 pg/ml) (20 min after the end of bleeding). Aortas taken from shocked rats (20 min after the end of bleeding) showed hypocontractility to Phenylephrine (PE; 1 nM-10 μM). Tacrolimus, an inhibitor of NFκB activation (100 μg/kg/i.v., 1 min after the beginning of bleeding), inhibited the loss of IκBα protein from the cytoplasm and prevented NF-κB binding activity in the nucleus. Furthermore tacrolimus increased survival rate (vehicle = 0% and tacrolimus = 80%, at 30 min after the end of bleeding), reverted the marked hypothension, decreased liver mRNA for TNF-α, reduced serum TNF-α (21 ± 5.3 pg/ml), and restored to control values the hyporeactivity to PE. Our results suggest that NF-κB plays an important role in acute HEM shock.

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CYTOKINE-INDUCED ENTEROCYTE-DERIVED NITRIC OXIDE INDUCES INTESTINAL MONOLAYER INJURY IN AN AUTOCRINE FASHION. R.M. Forsythe*, D.Z. Xu, Q. Lu* and E.A. Deitch UMDNJ-New Jersey Medical School, Newark, NJ 07103.

Cytokines, particularly interferon-γ (IFN-γ), have been shown to increase intestinal monolayer permeability, increase iNOS activity and increase production of nitric oxide. The goal of this study was to investigate the mechanism by which cytokines cause gut injury and to test the hypothesis that NO produced by enterocytes promotes gut injury in an autocrine fashion. **Methods**: Experiments were performed on rat intestinal epithelial cells (IEC-6) grown as a monolayer in a two-chambered system. First, monolayers were incubated with a cytokine mixture (CM) of IL-1β (10ng/ml), TNF-α (10ng/ml) and IFN-γ (250u/ml) and tested for permeability to phenol red and bacteria, plus NO_2/NO_3 production. Next, to determine if NO could mimic the effects of CM, cells were incubated with the NO donor SNAP (1mM) and tested for permeability. Then, to test if cytokine-induced monolayer permeability can be blocked by inhibiting IEC-6 NO production, IEC-6 monolayers were incubated with two NOS inhibitors (L-NMMA and L-NIL). **Results**: The cytokine mixture increased IEC-6 permeability to phenol red by 80.1% ($p<0.001$), increased NO levels from 1.5±0.6μM to 49.9±8.5μM ($p<0.01$) and increased BT by 1.5 log compared to controls ($p<0.001$). The NO donor, SNAP increased monolayer permeability to phenol red by 42.9% ($p<0.001$), as well as bacterial translocation ($p<0.001$). Lastly, inhibition of iNOS by L-NIL or L-NMMA prevented the cytokine-induced increase in monolayer permeability and bacterial translocation, supporting the role of enterocyte-produced NO in the pathogenesis of monolayer injury. **Conclusions**: Cytokine-induced disruption of monolayer barrier function appears to be secondary to enterocyte produced NO. This supports the hypothesis that NO produced by cytokine-stimulated enterocytes promotes injury in an autocrine fashion and highlights the fact that enterocytes can be a target as well as a producer of NO.

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PROGESTERONE IMPROVES CARDIOVASCULAR FUNCTION FOLLOWING TRAUMA-HEMORRHAGE AND RESUSCITATION.

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Although recent studies have shown that cardiovascular functions following trauma-hemorrhage are depressed in estrus females but not in proestrus females, the mechanism responsible for the protective effects during proestrus stage remain unknown. Since progesterone levels peak during the proestrus phase, our aim was to determine whether administration of progesterone following trauma-hemorrhage has any salutary effects on cardiovascular function. To study this, female Sprague-Dawley rats (150-175g) were ovariectomized 14 d prior to the experiments. Following a 5cm midline laparotomy (i.e., tissue trauma), they were bled to a mean arterial pressure of 40mmHg, until 40% of the maximal bleedout (MB) volume was returned in form of Ringer's lactate. Progesterone (25mg/kg BW) or vehicle was administered subcutaneusly and the animals were resuscitated with four times the volume of MB with Ringer's lactate over 1h. At 24 h after resuscitation, cardiac output (CO; ml/min 100g BW) was determined by a dye dilution technique. Cardiac contractility ($\pm dP/dt_{max}$ mmHg/sec) was also measured. Results are as follows:

	Sham	Vehicle	Progesterone
CO	43.4 ± 5.5	22.6 ± 1.2 *	34.9 ± 4.4
$+dP/dt_{max}$	8060 ± 873	5588 ± 719 *	8121 ± 710
$-dP/dt_{max}$	5311 ± 785	3405 ± 406 *	5535 ± 581

(means ± SEM , n= 7, * $p<0.05$ vs Sham and Progesterone, ANOVA and Student-Newman-Keuls test.)

Heart performance and CO were markedly depressed in animals at 24 h after trauma-hemorrhage and resuscitation. In animals receiving progesterone, CO and cardiac contractility were significantly improved and the values were not different than shams. Since progesterone treatment normalized cardiovascular function in ovariectomized females after trauma-hemorrhage and resuscitation, administration of this agent should be considered a novel adjunct for improving cardiovascular function under those conditions in ovariectomized and postmenopausal females. (Supported by NIH GM 39519).

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DO PERIPHERAL BLOOD MONONUCLEAR CELLS MIMIC THE SEXUALLY DIMORPHIC IMMUNE RESPONSE OF TISSUE IMMUNE CELLS FOLLOWING TRAUMA-HEMORRHAGE? C.P. Schneider*, T.S.A. Samy*, E.A. Nickel*, M.G. Schwacha, I.H. Chaudry. Center for Surgical Research, Brown University & RI Hospital, Middle House II, 593 Eddy Street, Providence, RI 02903

Although clinical studies have shown that peripheral blood mononuclear cell (PBMC) immune functions are depressed after traumatic injuries, it remains unclear if tissue-fixed immune cell functions are also depressed in patients under those conditions. Animal studies indicate that tissue-fixed immune cell functions are depressed in males (M), whereas they are maintained in proestrus females (F) after trauma-hemorrhage (TH). It remains unknown, however, whether PBMC exhibit a

similar sexual dimorphic immune response following TH. To study this, male and proestrus female C3H/HeN mice were sham operated (S) or subjected to trauma (i.e., midline laparotomy) and hemorrhagic shock (30±5 mmHg for 90 min) followed by adequate fluid resuscitation. Twenty-four hours after resuscitation, the animals were sacrificed and blood was collected. PBMC were isolated and *in vitro* functional capacity was assessed at the level of proliferative responses to anti-CD3 (1 μg/ml) and cytokine production (IL-6, TNF-α, IL-10) in response to LPS (10 μg/ml). A marked decrease in PBMC

	Proliferation cpm x 10^3	IL-6 (U/ml)	TNF-α (U/ml)	IL-10 (pg/ml)
M/S	13.5±2.5	347±58	8.1±2.4	78±25
M/TH	1.8±1.3*	49±18*	0.8±0.3*	19±17
F/S	8.7±3.6	319±45	10.6±2.9	104±19
F/TH	16.7±5.7†	143±31*†	3.3±0.4*†	50±12†

*N=6-7, Mean±SEM, ANOVA, * $p<0.05$ vs. S, † $p<0.05$ vs. M/TH.*

proliferation was observed in males following TH ($p<0.05$). In contrast, proliferation was significantly increased in females under such conditions. IL-6 and TNF-α release by PBMC was depressed in both genders following TH ($p<0.05$), however, the depression was significantly less in females. IL-10 release was unchanged in males following TH, but a significant decrease in females was observed under such conditions. Our study demonstrates for the first time that changes in murine PBMC function after trauma-hemorrhage corresponds/reflects the sexually dimorphic immune changes at the tissue level. Thus, our finding suggest that PBMC function is a good indicator of overall immune status. (*NIH GM 37127*).

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GLUTAMINE INDUCES HEAT SHOCK PROTEIN AND PREVENTS MORTALITY FROM ENDOTOXEMIA IN THE RAT. P. Wischmeyer, R. Wolfson*, M. Musch*, E. Chang*, M. Kahana*. Univ. Chicago, Chicago, IL 60637

Introduction: We have previously shown that glutamine (GLN), a non-essential amino acid, enhances cell survival *in vitro* against a variety of stressful stimuli through the induction of heat shock proteins (HSPs). Studies in animal sepsis models indicate that HSP induction decreases morbidity and mortality. The agents previously used to induce HSPs in these models are themselves toxic and therefore not practical for clinical application. Our aim was to determine if GLN induces HSP in the intact rat and protects against a lethal LPS injury. **Methods:** Male Sprague-Dawley rats weighing 250 g –350g were anesthetized using ketamine/xylazine. GLN or a lactated ringers (LR) control was administered via the tail vein. A GLN dose of 0.75 g/kg was utilized and tissues harvested 1-72 hours post-infusion. Tissues were analyzed for HSPs with Western blot using an antibody specific for inducible HSPs. Survival studies utilized 5mg/kg *Escherichia coli* lipopolysaccharide (LPS) injected concomitantly with GLN/LR infusion. **Results**: Glutamine infusion significantly increased hsp25 and hsp72 protein expression by 2-3 fold, appearing in less then 3 hours in lung and heart and within 12 hours in colon. A single dose of GLN was responsible for sustained HSP expression for 72 hours. Survival studies demonstrated 72% mortality in the LR group (n=7) and no mortality in the GLN group (n=7) ($p<0.02$). **Conclusion:** GLN infusion resulted in significant induction of HSP expression at the tissue level in an *in vivo* model. Furthermore, single dose GLN infusion given at the time of LPS injury prevented mortality in a rat model of lethal endotoxemia. These data suggest that GLN, which has previously been shown to improve infectious morbidity in clinical trials, is a non-toxic, clinically relevant inducer of HSP expression. This suggests the mechanism of GLN's protection against lethal endotoxemia may be a result of increased HSP expression.

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INTRALUMINAL NUTRIENTS ENHANCE GUT ISCHEMIA/REPERFUSION. INJURY

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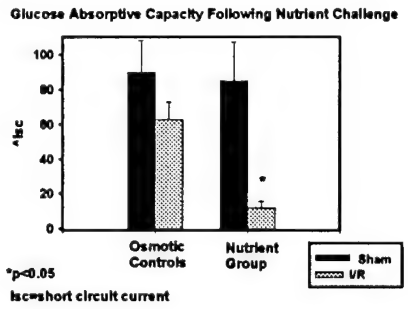
Early (<48 hrs) enteral nutrition (EEN) has been shown to improve patient outcome following trauma. However, severely injured patients who require shock resuscitation have persistent (>24 hrs) gut hypoperfusion and EEN may be deleterious by increasing the metabolic demand of an already stressed gut. We therefore hypothesize that intraluminal nutrients (absorbed by ATP-dependent transport) during gut ischemia/ reperfusion (I/R) enhance gut injury. At laparotomy, Sprague-Dawley rats had jejunal sacs exposed to 5mm glucose + 5 mm alanine (nutrient group) or 10 mm mannitol (osmotic control) followed by gut I/R (n=9) (superior mesenteric artery occlusion 60 minutes and reperfusion 30 minutes) or sham laparotomy (n=5). Jejunum was harvested for histologic studies and graded by a standard injury score or mounted in a Ussing chamber for determination of glucose absorptive capacity (GAC). Data are expressed as mean ± SEM. Histologically, the injury score was higher in the I/R nutrient group compared to I/R osmotic controls. GAC, estimated by the increase in Isc, did not differ between sham and I/R osmotic controls, however it was significantly depressed in the I/R nutrient group compared to all other groups. We conclude that intraluminal nutrients administered to a metabolically stressed gut enhance gut injury. Caution must be exercised when administering EEN to severely injured patients who require shock resuscitation.



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EFFECTS ON THE SECRETION OF METABOLIC REGULATING HORMONES (LEPTIN) AND POSTTRAUMATIC COMPLICATIONS IN BLUNT POLYTRAUMA PATIENTS M. van Griensven, K. Hucke*, A. Seekamp*, H.-C. Pape

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Multiple traumatized patients may develop SIRS or even MODS in the posttraumatic course. Likewise, it is known that gender differences and the related hormones play a role. Both, the inflammatory and the hormonal system interact on several biochemical levels. Among the metabolic regulating hormones, leptin

appears to play a pivotal role since patients surviving a septic insult display higher leptin levels. This might be dependent on the inhibiting effect of leptin on the hypothalamic-pituitary-adrenal axis. In this prospective study, we therefore investigated leptin concentrations in multiple traumatized patients to determine its role in the posttraumatic course. Leptin levels in normal controls amounted to 3.7 ± 1,4 ng/ml. These levels were significantly increased in patients without complications to 17.6 ± 4,2 ng/ml ($p<0.01$). Patients with complications displayed levels of 8.0 ± 2,1 ng/ml. Furthermore, a negative correlation between the levels of leptin and IL-6 was observed ($R^2=0.85$). Our data reveal sustained changes of leptin in blunt trauma patients, who develop posttraumatic complications. The inverse relationship towards IL-6 levels suggests that interactions between the inflammatory and the hormonal system occur. This inverse correlation to IL-6 serum levels may be explained by an indirect inhibitory effect of IL-6 on leptin secretion. This study provides an additional indication for the interaction between posttraumatic hormonal and immunological changes.

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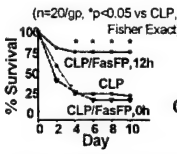
DELAYED BLOCKADE OF FASL RESTORES LYMPHOID IMMUNE FUNCTION, SUPPRESSES APOPTOSIS AND IMPROVES SURVIVAL IN SEPSIS. CS Chung, GY Song*, J Lomas*, HH Simms, IH Chaudry and A Ayala. Dept. Surgery, RI Hospital/Brown Univ. Schl. Med., Providence, RI 02903

Studies show that increased apoptosis (A_o) is associated with immune suppression and increased mortality in both septic patients and animals subjected to polymicrobial sepsis. In this respect, FasL has been shown to transduce the molecular signals coupled with induction of A_o in a variety of immune cell populations. However, while the potential role of FasL has been documented in a variety of pre-treatment scenarios, its contribution to the mortality as well as immune dysfunction seen in septic animals when given as a post-treatment remains unclear. To address the former issue, we conducted survival studies on C3H/HeN mice which received 5μg Fas receptor fusion protein (FasFP) or the saline vehicle (control) immediately (0h) or delayed (12h) after induction of polymicrobial sepsis by cecal ligation and puncture (CLP). The results indicate that only delayed administration (12h) but not 0h of FasFP showed a marked increase in survival. Subsequently, we examined the effect of FasFP treatment on the development of A_o and immunosuppression seen during sepsis. To study this, thymocytes (Thy), splenocytes (Spl) and peritoneal macrophage (pMϕ) from Sham-control, CLP or CLP/FasFP (12h-post) mice were harvested 24h post surgery. Spl were stimulated with Con A, while pMϕ were challenged with LPS and cytokine release as a functional index by ELISA. IL-2 and IFN-γ release by Spl is markedly depressed, while IL-10 release is augmented after CLP. However, treatment of CLP mice with FasFP restored IL-2 and IFN-γ production and prevented IL-10 release by Spl. Alternatively, depressed Mϕ cytokine release (IL-1, IL-6 and IL-12) in CLP mice was not restored with FasFP treatment. Similarly, while a marked increase in A_o was seen in septic Thy and pMϕ, FasFP treatment suppressed Thy but not pMϕ A_o. Taken together, these results indicate not only that delayed inhibition of FasL protects mice from septic mortality, but that this is associated with the preferential protection of lymphoid but not Mϕ function. (Supported by NIH GM 53209&57226)



	Spl Cytokine Release (ng/ml)			% A_o (PI stain)	
	IL-2	IFN-γ	IL-10	Thy	pMϕ
Sham	2.1±0.1	6.2±1.0	0.1±0.1	0.5±0.1	4.2±1.2
CLP	1.6±0.1*	2.3±0.2*	1.2±0.3*	8.2±0.6*	45.3±8.3*
CLP/FasFP	2.5±0.1#	4.2±0.4#	0.5±0.1#	5.3±0.8#	43.8±13.5

(n=5-6/gp, *p<0.05 vs Sham; #p<0.05 vs CLP, Mann Whittney U)

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ROLE OF KUPFFER CELLS AND NEUTROPHILS FOR THE REGULATION OF HEME OXYGENASE-1 GENE EXPRESSION IN THE LIVER UNDER STRESS CONDITIONS.
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Heme oxygenase (HO)-derived carbon monoxide plays a pivotal role for maintenance of liver blood flow under stress conditions (1). Gene expression of the stress-inducible isoenzyme HO-1 can be transcriptionally activated through oxygen free radicals (OFR). In the present study, we investigated the role of Kupffer cells and neutrophils as paracrine modulators of hepatocellular HO-1 gene expression in a rat model of hemorrhage and resuscitation. Sprague Dawley rats (n=6-10/group) were anesthetized (pentobarbital, 50mg/kg) and subjected to hemorrhagic shock (MAP: 35-40mmHg for 60 min) or a sham protocol. Based on the time course of HO-1 gene expression, the effect of antioxidants, Kupffer cell blockade ($GdCl_3$; 10mg/kg; 24h prior to hemorrhage), or neutrophil depletion (vinblastine; 0.5mg/kg, 5days prior to hemorrhage) on induction of the HO-1 gene was assessed at 5hours of resuscitation by standard Northern and Western blot analysis. Hemorrhage and resuscitation induced HO-1 gene expression with a maximum at 5h of resuscitation (11.4 fold over control). Kupffer cell blockade and antioxidants abolished HO-1 mRNA and protein induction after hemorrhage, while neutrophil depletion failed to affect hepatocellular HO-1 induction (HO-1 mRNA arbitrary densitometric units: vehicle shock 10.2±5.4, $GdCl_3$ shock 3.6±2.1 and vinblastine shock 9.3±2.1). Since OFR are the predominant second messengers for HO-1 gene expression, our data suggest that Kupffer cells but not neutrophils induce a hepatocellular oxidative stress response after hemorrhage and resuscitation. OFR released by Kupffer cells serve as paracrine regulators of a hepatocellular stress gene which is necessary to maintain liver blood flow and integrity under stress conditions (1).

(supported by DFG grant (Ba1601/1-2). (1) Bauer M. et al. *Am J Physiol* 1996; **271**:G929-G935.

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EXPRESSION PATTERN AND REGULATION OF HEME OXYGENASE-1/HEAT SHOCK PROTEIN 32 IN HUMAN LIVER CELLS.
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Heme oxygenase- (HO-) 1 is highly inducible by oxidative or heat stress. Studies in rodents suggest that the HO/carbon monoxide pathway subserves a similar function in oxidative stress models as does the nitric oxide synthase/nitric oxide pathway in models of liver inflammation. The present study investigated expression pattern and regulation of HO-1 in human liver biopsies and cell systems by Northern and Western blot analysis, and immunohistochemistry. Only Kupffer cells

expressed HO-1 protein constitutively. However, HO-1 was inducible in hepatocytes and vascular tissue under pathological conditions, e.g. associated with fatty degeneration or liver malignancies. Regulation of HO-1 gene expression was further studied in HepG2 cells and freshly isolated peripheral blood mononuclear cells (PBMC) as models of parenchymal and non-parenchymal liver cell populations, respectively. HO-1 was inducible in HepG2 cells and PBMC by glutathione depletion and $CoCl_2$ but not by heat shock. Pyrrolidine dithiocarbamate, an inhibitor of nuclear factor κB- (NF-κB-) dependent gene expression, dose-dependently decreased HO-1 mRNA transcripts in PBMC subjected to oxidative stress while slightly increasing HO-1 gene expression in HepG2 cells. HO-1 induction upon oxidative stress was attenuated in HepG2 cells by cycloheximide and dexamethasone. NF-κB seems to play a significant role in HO-1 induction in human mononuclear cells while our data are consistent with a role for AP-1 in in human HepG2 hepatoma cells. These data suggest a differential regulation of HO-1 gene expression in parenchymal and non-parenchymal human liver cells and may provide a topographic basis for the understanding of the role of the heme oxygenase/carbon monoxide pathway in human liver disease.

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ENDOTOXIN MEDIATED BLOCKADE OF PREGNANE X RECEPTOR TRANSLOCATION: EFFECTS ON HEPATIC CYTOCHROME P-450.

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Cytochrome P-450 (CYP) isozyme activity is significantly altered following endotoxin (LPS) administration. We have previously shown that the metabolism of lidocaine (LC) is significantly decreased following acute endotoxic shock in the rat. The mechanism for the reduced LC metabolism is unclear, but is likely due to reduced blood flow and hepatocellular dysfunction. An orphan nuclear receptor, termed the pregnane X receptor (PXR), mediate the induction of CYP3A, which is the most abundant CYP enzyme that is responsible for the metabolism of the majority of drugs, including LC. Northern blotting has shown that several prototypical CYP inducers markedly affect the accumulation of rat PRX mRNA, which likely in turn affects CYP3A induction. This study was conducted to determine if LPS affects the expression of PXR, which could explain the altered metabolism of LC during endotoxic shock. A peptide derived from rat PXR was synthesized and conjugated with the keyhole limpet hemocyanin. An antibody was raised against the conjugated peptide and subjected to affinity chromatography. This antibody detected a protein only in the PXR-transfected COS-7 cells but not in the control cells, suggesting that this antibody is highly specific. Male Sprague-Dawley rats were treated with LPS (46 mg/kg, i.p.) and sacrificed at various times up to 8 h. After 8 h total CYP and CYP3A1 levels were decreased. Nuclear and cytosolic staining of PXR in liver fractions was present in control rats; however, in LPS treated rats, PXR staining was undetectable in the nucleus but was increased in the cytosol. These results suggest that LPS-mediated blockade of PXR-translocation contributes to the alteration of LC metabolism.

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FLAGELLIN, A NOVEL MEDIATOR OF GRAM NEGATIVE BACTERIA - INDUCED SHOCK.

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Flagellin (Flg) is the monomeric subunit obtained from the flagella of Gram negative (G^-) bacteria. In addition to their role in motility, flagella may also act as a virulence factor. In this study, we have explored the potential role of Flg as a mediator of G^- bacteria-induced inflammation and shock. *In vitro,* purified Flg (1μg/ml) from *Salmonella dublin* rapidly (30 min) induced NFκB activation (electromobility gel shift assay) and IκBα degradation (western analysis) in the human intestinal cell line Caco-2BBe cells. Flg also induced iNOS protein expression (western analysis) and NO production (Griess reaction) in IFN-γ primed CACO-2BBe and DLD-1 cells (another human intestinal cell line) exposed to 1μg/ml Flg for 16 hours. *In vivo*, the intraperitoneal injection of 400μg/kg Flg to C57BL/6 mice increased the levels of plasma TNF-α, MIP-1 α, IL-6, IL-12 p40, IL-10 and nitrate after 2-24 hours. A similar pattern was noted in lipopolysacharride (LPS)-resistant mice (C3H/HeJ), indicating that the effects of Flg were not due to LPS contamination. Finally, the intravenous administration of 10 mg/kg recombinant *S.muenchen* Flg to anesthetized mice (LPS resistant C3H/HeJ and LPS sensitive C3H/HeOuJ and Balb/C) induced a progressive hypotension leading to death in 2-4 hours, which was similar in all the strains of mice. Overall, these data indicate that Flg may represent a novel, unrecognized mediator of G^--bacteria-mediated systemic inflammation and shock.

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CD16 BLOCKADE IN POLYMICROBIAL SEPSIS INCREASES HEPATIC BUT NOT PULMONARY NEUTROPHIL SEQUESTRATION

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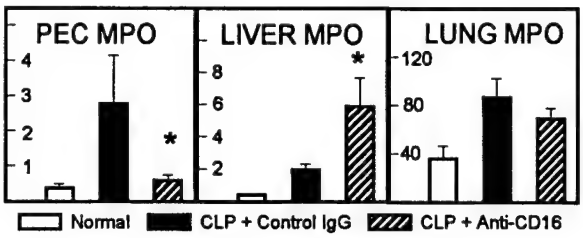
Polymorphonuclear leukocyte (PMN) influx into tissues remote from the site of infection is a key event in the pathogenesis of multi organ failure in sepsis. Pharmacologic blockade of P-selectin, CD18 and CD11b using monoclonal antibodies prevents PMN migration into the peritoneum during polymicrobial sepsis from cecal ligation and puncture (CLP). Additionally, PMN influx into liver and lung is increased over CLP alone. CD16/CD32 has multiple functions in the inflammatory response, including regulation of phagocytosis, bacterial killing, and activation of PMN and other leukocytes. However, its role in PMN infiltration during sepsis is unknown. The purpose of this study is to examine the role of CD16/CD32 in organ PMN accumulation during polymicrobial sepsis. CLP was performed in Swiss Webster mice after intraperitoneal injection of either anti CD16/CD32 or isotype control monoclonal antibody. Four hours later the mice were sacrificed, and liver, lung and peritoneal exudate cells (PECs) were harvested. PMN accumulation was determined by myeloperoxidase (MPO) assay. PMN transmigration was blocked in the peritoneum, but increased in

the liver. In contrast to previous blocking studies with other antibodies, there was no effect on lung MPO or peripheral leukocyte count. These results suggest that CD16 plays a role in the migration of neutrophils, and has a specific effect in the liver.



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ADENOSINE-MEDIATED ALTERATIONS IN TESTICULAR CYTOKINE AND TESTOSTERONE PRODUCTION A.M. Clark*, Z.S. Jonjev*, T. Diemer, D.B. Hales, J.L. Ferguson, W.R. Law, and H.B. Bosmann
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The effects of adenosine on testicular cytokine and testosterone production in the rat are unknown. Adenosine is known to modulate peritoneal and alveolar macrophage cytokine production. Testicular macrophage cytokines have been implicated in altered steroidogenesis after LPS challenge. Our laboratory hypothesizes that endogenous adenosine can alter testosterone (T) production via modulation of testicular macrophage cytokine production. Rats (n=5) received LPS ip (2 mg/kg in 4 mg/ml solution) or an equivalent volume of saline (n=5). Two hours after injection, rats were euthanized and the testis were removed. Leydig cells (LC) and interstitial macrophages (M) were isolated using our laboratory's continuous Percoll gradient method. Purified co-cultures of LC and M were generated and three treatment groups were plated: PBS (control), 10μM 8-sulfophenyltheophylline (8-SPT; adenosine receptor antagonist), and 50μM pentostatin (PNT; adenosine deaminase inhibitor). After twenty-four hours of *in vitro* treatment, media was recovered and analyzed for T (RIA) and TNF-α (ELISA) concentrations. Concentrations of T were significantly lower in control media from LPS-treated rats compared to saline treated rats (corrected for cell numbers). In cells from LPS rats, blockade of adenosine receptors (8-SPT) resulted in consistently higher T concentrations, while inhibition of adenosine deaminase consistently suppressed T. Similarly, adenosine receptor blockade (8-SPT) resulted in higher TNF-α (138±21% of PBS treatment) while inhibition of adenosine deaminase suppressed TNF-α (4±5%). No significant effects of 8-SPT or PNT (compared to PBS) were seen in LC/M cells from saline rats. These data suggest a modulatory role for adenosine in regulating Leydig cell steroidogenesis in the presence of activated macrophages.

Pentostatin was a generous gift from Supergen, Inc.

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POSTTRAUMATIC DISTURBANCES OF HUMORAL BONE FACTORS IN TRAUMA PATIENTS
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Traumatic brain injury (TBI) combined with fractures of long bones or large joints is often associated with enhanced ostogenesis (early fracture healing accompanied by hypertrophic callus formation and/or heterotopic ossifications). However, it remains unknown which humoral factors cause enhanced osteogenesis in patients with traumatic brain injury. The aim of our study was, therefore, to reveal if post-traumatic changes of hormone levels in trauma patients could be associated with different injuries. 20 patients were divided in two groups: patients with severe brain injury and bone fractures (n = 9) and those with only fractures (n = 11). Blood samples were taken on day 0, 1, 3, 5, and 7 after trauma and PTH (parathyroid hormon), CT (calcitonin), OC (osteocalcin), PICP (carboxyterminal propeptide of type I procollagen), ICTP (carboxyterminal piridinolin cross linked telopeptide of type I collagen), AP (bone isoenzyme alkaline phosphatase), Ca (calcium), and P (phosphate) levels were determined.

	ICTP (ug/l)		PTH (pg/ml)		OC (umol/l)	
	BI+Fra.	Fra.	TBI+Fra.	Fra.	TBI+Fra.	Fra.
d0	6,2* ± 1	3,3 ± 0,3	128*± 17	44 ± 6	2,1* ± 0,	5,5 ± 0,6
d1	7,1* ± 2	4,1 ± 0,3	38 ± 12	45 ± 3	1,5* ± 0,	4 ± 0,4
d3	15,9* ± 5	4,3 ± 0,4	39 ± 2	33 ± 5	1,5* ± 0,	3,9 ± 0,4
d5	16,1* ± 5	4,4 ± 0,3	46 ± 20	22 ± 4	1,9* ± 0,	4,2 ± 0,8
d7	16,3* ± 6	4,7 ± 0,3	26 ± 9	30 ± 6	1,7* ± 0,	5,1 ± 0,9

Student -t test, *p < 0.01 vs fracture

Thus, ICTP levels in the TBI group were significantly higher over the whole period, PTH levels in the TBI group were significantly enhanced on the day of trauma, OC levels in the TBI group were significantly lower than in the fracture group. Hence, ICTP, PTH, and OC seems to be humoral factors which might influence fracture healing and heterotopic ossifications.

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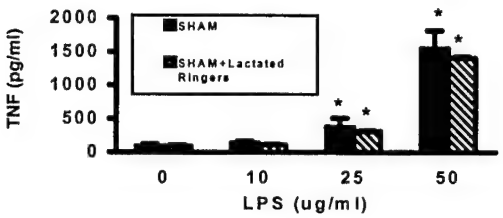
EFFECTS OF LACTATED RINGERS ON CARDIOMYOCYTE TNF-α SYNTHESIS. J.W. Horton, D. Maass* and J. White*. UTSWMC, Dallas, TX 75390-9160.

Previously we described that burn trauma increases TNF-α secretion by cardiomyocytes, suggesting that cardiac synthesis of inflammatory cytokines contributes to postburn cardiac contractile abnormalities. However, this previous work used a burn model that included lactated Ringer's (LR) fluid resuscitation. Since recent studies have raised questions regarding the cellular consequences of crystalloid resuscitation from trauma, we chose to examine effects of LR administration in the absence of burn trauma on cardiac function and myocyte TNF synthesis. Non-burned Sprague Dawley rats were given LR solution (4 ml/kg/40% burn based on our previous % burn) or no fluid; 24 hrs after initiating LR, hearts were used to assess function (Langendorff) or to prepare myocytes (collagenase digestion). Myocytes were plated ($5x10^5$ cells/well) and stimulated with LPS (Difco Labs) at a concentration of 0, 10, 25, or 50 μg for 18 hrs, and supernatant TNF concentration (ELISA, Endogen rat TNF-α) was measured. Compared to controls, LR infusion in non-burned rats did not alter cardiac function (LVP: 95±2 vs 98±6 mmHg; +dP/dt: 2202±48 vs 2217±136 mmHg/sec; -dP/dt: 1890±60 vs 1917±125 mmHg/sec). Myocytes harvested from both LR treated and control rats responded similarly to LPS challenge with dose-dependent increases in TNF secretion (Figure). These data confirm that large volume LR infusion in control rats does not trigger either a cardiac inflammatory

cytokine response nor alter cardiac function. Supported by NIH Grant (GM 21-681-35).

**indicates significant difference from zero (no LPS).*



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MICROVASCULAR EFFECTS OF ORAL IL-6. FM Rollwagen, *Y-Y Li, *ND Pacheco, *EJ Dick and *Y-H Kang. USUHS, Bethesda, MD 29814, Air Force Research Laboratory, Brooks AFB, TX 78235 and NMRC, Bethesda, MD 20814

Ischemia reperfusion (I/R) injury is a serious complication of surgery or trauma. Hemorrhage and subsequent physiological changes initiate a downward spiral of oxygen free radical damage, lipid peroxidation and loss of cellular Ca^{++} control leading to cell death. The small intestine, particularly the ileum, is susceptible to I/R damage, since reperfusion of the gut fails to take place when peripheral blood pressure is restored. Gut damage, leading to contamination of the host by LPS and other toxic substances may be responsible ultimately for multiple organ failure and death. Oral administration of Interleukin (IL)-6 has been shown to reduce I/R injury following hemorrhagic shock in mice and rats. We examined the intestinal circulation by electron microscopy in this model using horseradish peroxidase (HRP) as a tracer. Given iv, HRP was found between intestinal epithelial cells in normal and hemorrhaged mice fed IL-6, showing that the circulation was patent, and that the label reached the ileum. When the tracer was given intralumenally, in similarly treated mice, the intestine excluded the tracer at the zonula occludens. In mice given saline following hemorrhage, the tracer administered iv could not be found in the circulation at the epithelial level. Tracer administered intralumenally, however, penetrated the epithelium and could be found in the blood vessels of the submucosa. These data suggest that oral IL-6 restores intestinal circulation following hemorrhage, and in so doing, prevents leakage of intestinal contents into the interior.

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REMOVAL OF FATTY ACIDS IMPROVES COUPLING OF EX-VIVO MYOCARDIAL GLYCOLYTIC FLUX TO GLUCOSE OXIDATION AFTER HEMORRHAGE

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Increased glucose oxidation : glycolysis ratio improves cardiac efficiency (CE). This study tested if the absence of fatty acids (FFA) improved these parameters after hemorrhagic shock. Methods: Shock was induced in non-heparinized, ketamine / xylazine-anesthetized rats by blood removal to yield a MAP ~30 mm Hg for 60 min. Hearts were excised and perfused for in working mode with either 11 mM U-^{14}C/5-^{3}H glucose + 0.4 mM palmitate or 11 mM U-^{14}C/5-^{3}H glucose only. Measurements included CE (work/O_2 consumption, %), glycolytic flux (nmol/min/g dry mass) and glucose oxidation (glucox, nmol/min/g dry mass). Data were compared after 60 min perfusion by t-test, n=5-7/group. Results: Shocked hearts perfused with glucose + palmitate demonstrated similar glycolytic flux (7256±1316, shock vs. 8284±850, sham), and glucox (213±76 vs. 365±188) which resulted in unchanged glucox /glycolysis (4±1 vs. 4±1) but tended to have lower CE (12.1±0.8 vs. 13.9±0.6). However, with glucose-only perfusion, glycolysis was significantly decreased in shocked hearts compared to shams (4392±1504 vs. 12878±2070, $p < 0.05$), and glucox was stimulated (1043±227 vs. 512±157). This resulted in increased glucox/glycolysis (33±1% vs. 6±2%, $p < 0.05$) and tendency for higher CE (12.7±1.4% vs. 10.1±0.9%). Conclusions: Therapy designed to stimulate glucose uptake and decrease FFA exposure after acute hemorrhagic shock will increase myocardial coupling of glucose oxidation to glycolytic flux and has the potential to increase cardiac efficiency.

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SEPSIS GENE EXPRESSION PROFILING: MURINE SPLENIC COMPARED TO HEPATIC RESPONSES DETERMINED USING cDNA MICROARRAYS. JP Cobb, WD Shannon*, JJ Morrissey*, Y Qiu*, JM Laramie*, IE Karl*, TG Buchman, RS Hotchkiss. Cellular Injury and Adaptation Laboratory, Washington University, St. Louis, MO 63130.

We hypothesized that analysis of global changes in gene expression would provide novel insights into the organ-specific pathways that regulate the inflammatory response to sepsis. Male C57BL/6 mice were assigned to laparotomy either with or without cecal ligation and puncture (CLP). After 24 h, 3 liver and 3 spleen specimens were obtained from CLP and SHAM groups. Total RNA was isolated from homogenized tissue using TRIzol®. RNA was reverse transcribed to make cDNA using Clontech Atlas™ cDNA Expression Array kits. Radioactive cDNA target from each sample was hybridized with Atlas™ microarrays spotted with cDNA specific for 588 murine genes. Profiling was performed on mean gene expression intensities using AtlasImage™ software and cluster analysis algorithms. Compared to SHAM, CLP induced significant changes in gene expression in both spleen and liver (>2 fold change in 69 splenic and 58 hepatic genes). Moreover, 73 genes were differentially expressed >2 fold in spleen *vs.* liver after CLP. For example, organ-specific differences were identified in the expression profiles of genes associated with inflammatory stress (*e.g.*, NF-^{K}B p105), oxidative stress (*e.g.*, GSH transferase), heat stress (*e.g.*, HSP 60), and apoptosis (*e.g.*, FasL), and others (see Table). We conclude that broad-scale profiling provides insight regarding organ-specific changes in

gene expression after CLP, implicating regulatory pathways that control apoptosis, cell signalling, and response interactions.

gene (6 of 73)	septic spleen/liver gene expression ratio
laminin receptor 1	10
IFN-γ receptor	4.76
GM-CSF receptor	3.12
ICAM-1	2.70
integrin β7	0.27
urokinase plasminogen activator	0.17

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GENETIC DISRUPTION OF POLY(ADP-RIBOSE) SYNTHETASE REDUCES GUT DYSFUNCTION AND DISTANT ORGAN DAMAGE IN MESENTERIC ISCHEMIA-REPERFUSION INJURY

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Mesenteric ischemia-reperfusion (MIR) is associated with the formation of nitrogen- and oxygen-centered free radicals, which contribute to the development of gut injury in this setting. In particular, oxidant-mediated DNA damage leads to the activation of the enzyme poly(ADP-ribose) synthetase (PARS), resulting in ATP depletion and cell necrosis. Here, we have evaluated the role of PARS in a murine model of MIR, using $PARS^{+/+}$ and $PARS^{-/-}$ animals. Mice were exposed to a 30 min occlusion of the superior mesenteric artery, followed by 3 h reperfusion. At the end reperfusion, a segment of ileum was taken to assess gut permeability, by measuring the mucosal to serosal clearance of fluorescein-dextran (FD-4) in everted gut sacs incubated *ex vivo*. Samples of lung and liver were harvested for the determination of neutrophil infiltration (myeloperoxidase, MPO), and lipid peroxidation (malondialdehyde, MDA). **Results**:

Variable	PARS +/+	PARS-/-
FD-4 clearance[a]	243±49	31±4 *
Lung MDA[b]	0.52±0.02	0.36±0.03 *
Liver MDA[b]	2.63±0.77	0.70±0.09 *
Lung MPO[c]	203±33	157±21
Liver MPO[c]	13±3	2±1 *

M±s.e.m. [a] nL/min/cm^2 [b] nmol/mg [c] mU/mg protein

* $p<0.05$ $PARS^{-/-}$ vs $PARS^{+/+}$

$PARS^{-/-}$ animals had a marked reduction of intestinal hyperpermeability following MIR, as well as a lesser degree of neutrophil infiltration in the liver and of lipid peroxidation in the lung and liver. These data support a mechanistic role of PARS in the pathophysiology of mesenteric ischemia-reperfusion injury.

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POST HEMORRHAGIC SHOCK MESENTERIC LYMPH UPREGULATES E-SELECTIN EXPRESION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC).

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We have previously shown that mesenteric lymph from shocked rats contributes to shock-induced lung injury. One mechanism may be that lymph causes an upregulation of endothelial cell adhesion molecules. Thus, the ability of post shock mesenteric lymph to induce expression of E-selectin in HUVECs was tested. **Methods:** Mesenteric lymph was collected from male Sprague-Dawley rats that were subjected to sham or hemorrhagic shock (30mmHG for 90 minutes) and volume resuscitated. HUVECs seeded on Matrigel coated 96-well plates and grown to confluence were incubated with medium, post-shock or sham-shock humoral lymph (5% concentration) for 4 hours at 37C, following which the HUVECs were fixed. ELISA was performed using 1ug/ml anti E-selectin antibody and a second ALKPHOS-conjugated anti-mouse antibody. The amount of E-selectin expression was detected through a colorimetric method using pNPP and normalized for cell viability and number. Cell viability was assessed using a mitochondrial tetrazolium assay (MTT). **Results:** Lymph from the hemorrhagic shock rats increased E-selectin expression when compared to sham-shock or medium only groups. The shock lymph increased HUVEC E-selectin expression 2.7±1.5 fold compared to medium, and over 2 fold compared to sham shock lymph ($p<0.05$, nonparametric t-test). There was no difference in upregulation of E-selectin between HUVECs exposed to sham-shock lymph or medium alone. **Conclusion:** The results of this study indicate that post-shock mesenteric lymph increases E-selectin expression in HUVEC monolayers when compared to sham shock lymph or medium alone. These findings suggest that gut derived factors contained in the mesenteric lymph contribute to upregulation of endothelial cell adhesion molecules and may in this way contribute to shock-induced organ injury.

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A TIME COURSE STUDY OF THE PROTECTIVE EFFECT OF MESENTERIC LYMPH DUCT LIGATION ON HEMORRHAGIC SHOCK-INDUCED PULMONARY INJURY AND THE TOXIC EFFECTS OF SHOCK LYMPH ON HUVEC CELL MONOLAYER PERMEABILITY.

E.A. Deitch, C. Adams*, Q. Lu, DZ Xu, NJMS, Newark, NJ 07103

Introduction: We have documented that mesenteric lymph duct division protects against shock-induced lung injury when tested 3 hrs post shock and that lymph collected at 3 hrs post-shock increases endothelial cell (EC) monolayer permeability. However, it is not known whether the protective effect of lymphatic division (LD) persists or whether lymph collected at other time points post-shock is toxic. Thus, we tested the protective effects of LD on lung permeability at 6, 12 and 24 hrs post-shock as well as the ability of lymph collected before during and hourly (up to 6 hrs) after shock to increase EC monolayer permeability. **Methods:** 3,6,12, or 24 hrs after sham or actual shock (30 mm Hg for 90 min) lung permeability was measured using Evans blue dye in rats subjected to sham or actual LD. In separate experiments, the ability of lymph collected from rats subjected to shock or sham shock to increase HUVEC monolayer permeability to a 40 kD dextran rhodamine permeability probe was tested. Lymph was tested at 10% and 1% concentrations. **Results:** Hemorrhagic shock induced a 3-4 fold increase in lung permeability compared to sham-shock rats when tested at 3,6,12 or 24 hrs post-shock. LD prevented this increase in lung permeability at each of these time points. Sham shock lymph did not increase HUVEC permeability, while lymph from the shocked rats did, whether tested at 1% or 10%. Lymph samples collected during the shock period and hourly for 6 hours post-shock all increased HUVEC permeability, however the greatest relative increase in

HUVEC permeability was observed in the 3 and 6 hr post-shock samples. **Conclusions:** Lung injury after hemorrhagic shock appears to be caused by toxic factors carried in the mesenteric lymph and factors capable of increasing HUVEC permeability initially appear in the lymph during the shock period and increase over time.

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LBP PROMOTES BACTERIAL KILLING OF SILVER SULFADIAZINE RESISTANT P. AERUGINOSA IN INFECTED BURN WOUNDS. RD. Klein*, L. Steinstraesser*, A. Aminlari*, WH. Alarcon*, V. Khilanani*, BKS. Varma*, HY. Zhang*, GL. Su* & SC. Wang, Univ. of Michigan, Ann Arbor, MI 48109

Skin can mount an inflammatory response following thermal injury that is characterized by production of cytokines, recruitment of immune cells, containment of invading organisms and clearance of noxious substances from the wound. Lipopolysaccharide binding protein (LBP) is a 60 kD glycoprotein capable of coordinating all four functions and we have previously found evidence of LBP production within burn wounds. In prior experiments we also observed that burn wounds expressing lower than usual local levels of LBP were more likely to be significantly infected. Given these findings, we sought to determine the effect of recombinant LBP on burn wounds infected with a silver sulfadiazine resistant strain of *P. aeruginosa*. Depilated male Sprague Dawley rats underwent a 10% body surface area partial-thickness burn followed by wound innoculation ($1x10^6$ b/ml) with a strain of *P. aeruginosa* resistant to silver sulfadizine. Occlusive dressings were applied to prevent environmental contamination. After 48 h, rats were randomized into three groups and received intradermal injections of either: 1) 20% recombinant LBP, 2) 20% conditioned media (control) or 3) gentamycin. Wounds were harvested aseptically 24 h later and bacterial counts obtained by plating wound homogenates on Pseudomonas isolation agar. Statistically significant reductions in *P. aeruginosa* counts were found in the LBP treated group as compared to control. Counts were found to be similar between the gentamycin and LBP groups. These studies demonstrate that recombinant LBP can promote the clearance of *P. aeruginosa* from infected burn wounds and highlight the crucial role that LBP plays in host defenses against bacterial infection. Modulating local levels of LBP may therefore be considered for the treatment of burn wounds infected with organisms resistant to conventional topical antibiotics.

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DISTRIBUTION OF MONOHYDROXY FATTY ACIDS (MHA) IN MURINE SKIN FOLLOWING THERMAL INJURY. K. Langen*, M. Awad*, R. Shankar, R. Gamelli and S.B. Jones. Loyola Univ. Med. Center, Maywood, IL 60153.

Following burn trauma, loss of dermal protection and a decline of cell mediated responses contribute to morbidity and mortality. Metabolites of arachidonic and linoleic acid (MHFA) are known to serve as important signaling molecules and are often involved in immune responses. The present work determined if the production of these mediators was altered in skin following thermal injury. Male BDF1 mice were divided into sham, burn and burn infection groups. Burn and burn infection groups underwent a 15% full thickness surface scald. Animals in the burn infection group were then inoculated with 1000 CFU of *Pseudomonas aeruginosa* at the burn site. Sham-treated mice were subjected to room temperature water. Animals were killed 72 hrs after burn trauma and skin samples (8 mm punch) taken and stored in liquid nitrogen. Tissues were homogenized and MHA were extracted using SepPak C 18 columns. MHA were separated by HPLC on a straight phase silica column employing hexane-isopropanol- acetic acid as the mobile phase. Results indicate consistent concentrations of 12 and 15 HETE and 9 and 13 HODE in murine skin samples. With burn trauma 12 HETE levels were elevated in mid-burn areas (539±207 [X±SEM] vs 178±83 ng/g wet wt sham) but this was much less when infection was combined with burn (288+57). Changes in 9 HODE values reflect a similar pattern of elevation with burn alone (1039±629 midburn vs 641±127 sham) and such elevations were also less with burn plus infection (468±61 midburn). 13 HODE was elevated following burn (628±200 midburn vs 281±75 sham) but unlike 12 HETE and 9 HODE, such values were not markedly reduced with burn plus infection (587±212 midburn area). In contrast, 15 HETE following burn (872±300) was less than sham levels (1022±105) and was reduced further when infection was combined with burn (643±153). Thus, burn trauma results in elevations in 12 HETE, 9 and 13 HODE but the first two of these three appear to be reduced by infection. 15 HETE is reduced by burn trauma with a greater reduction following burn plus infection. Results are similar to those seen in one day following burn and burn plus infection and suggest that such changes in MHA may play a role in wound healing. Supported by MH53562 (SBJ) and GM56424 (RS).

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WHICH RECEPTOR MEDIATES PROSTAGLANDIN E_2 (PGE_2) / THROMBOXANE A_2 SYNERGY? F. Mahzari*, K. Wright*, C. Faulk*, R. Turnage. Univ. of Texas Southwestern Med. Sch. & Dallas VA Med. Ctr., Dallas, TX

Previous studies in our laboratory have demonstrated that PGE_2 significantly enhances the effects of TxA_2 on lung microvascular permeability. The purpose of this study was to determine which PGE_2 receptor (EP1,EP2, or EP3) contributes to this effect. The lungs of anesthetized Sprague-Dawley rats were excised and perfused *ex vivo* with Krebs-Henseleit buffer containing one of the selective EP receptor agonists, sulprostone (EP3>EP1; 10^{-9} M), misoprostol (EP2 & EP3; 10^{-9} M), 17 phenyl-trinor prostaglandin (EP1 > EP3; 10^{-9} M), 11 deoxy PGE_2 (EP2) with or without the TxA_2 receptor agonist U-46619 (7×10^{-8}M). 30 min. later pulmonary microvascular permeability was assessed by measuring the capillary filtration coefficient (K_f) using a gravimetric technique. Data are expressed as mean ± SEM and analyzed by Mann-Whitney U test & ANOVA.

N ≥ 4 per group	Receptor selectivity	Agonist Alone	Agonist + U-46619
Perfusate	-	0.9 ± 0.2	3.2 ± 0.5*
PGE_2	-	2.7 ± 0.8	4.9 ± 0.7*
17-phenyl	EP1>EP3	2.87 ± 0.31	208 ± 88*#
Misoprost	EP2&EP3	1.7 ± 0.33	18.5 ± 6.5*
Sulproston	EP3>EP1	3.3 ± 0.51	81 ± 48*
11-deoxy	EP2	7.19 ±	150 ± 71.3*#

Kf expressed as gm/min/mmHg/100 gm wt; Rt expressed as mmHg/ml/min/100 gm wt * $p < 0.05$ vs agonist alone; #$p < 0.05$ vs perfusate + U-46619 alone

The greatest synergistic effect on U-46619-mediated increases in K_f were seen with 17-phenyl-trinor prostaglandin and 11-deoxy PGE_2. These agonists activate the EP1 and EP2 receptors (with lesser effects on EP3). Misoprostol and

sulprostone had lesser effects on microvascular permeability. These data suggest that the synergistic effect of PGE_2 on TxA_2 induced pulmonary microvascular permeability is mediated, at least in part, via the EP1 and EP2 receptors, and perhaps, the EP3 receptor.

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MITOGEN-ACTIVATED PROTEIN KINASES (MAPK) IN THE ICU: POTENTIAL PROGNOSTIC FACTORS

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As to date, no therapeutic intervention has demonstrated a significant impact on the outcome of patients with MODS and ARDS. Most attribute this to an inability to identify the patient at risk. Current hypotheses regar ding the etiology of these syndromes embrace a 2 hit phenomenon, in which an initial stimulus primes for an aberrant reaction to a second stimulus. The family of MAPK is known to mediate endotoxin signaling. Hence, we investigated the MAPK status of at-risk trauma patients. Methods: Human trauma subjects necessitating intubation underwent bronchalveolar lavage(BAL) on days 3 and 5 postinjury. Macrophages were isolated by adherence and stimulated with endotoxin. Total cell protein was extracted and subjected to western blot analysis. Active p38 and ERK were measured using a phospho-specific antibody. The status of these parameters was compared to the response of monocytes from healthy donors and correlated with the patient's clinical status. Results: Monocytes from normal subjects demonstrated low basal p38 and ERK activity; LPS stimulation induced a 10-fold increase in MAPK activity within 30 minutes. Trauma subjects with Day 3 responses similar to normal controls were extubated prior to Day 5. A proportion of patients demonstrated high basal levels of p38 and ERK that was refractory to further stimulation with LPS; they remained intubated. By day 5 a significant proportion of these patients had reacquired normal LPS-induced p38 and ERK activation. Conclusion: Trauma patients demonstrate a progression in MAPK basal and LPS-induced activity. A normal p38 and ERK response on day 3 appeared to predict resolution of the stress response. Elevated day 3 basal p38 and ERK activity, refractory to LPS stimulation, predicted prolonged ICU support. Identification of this "primed" patient prior to the second insult might enable therapeutic interventions to have an impact on survival.

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PROTEGRIN-1 ENHANCES BACTERIAL KILLING IN THERMALLY INJURED MURINE EPIDERMIS

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Septic complications and the emergence of drug resistant microbes represent serious risks to the burn patient. Recently, a class of naturally occurring peptides has been discovered which have potent and broad-spectrum antimicrobial activity. One such antimicrobial peptide is Protegrin-1 (PG-1) which is particularly attractive for therapeutic use in human burns. Unlike defensins, PG-1 retains broad anti-microbial activity at the physiologic salt concentration, which is found in burn wounds. The objective of this study was to examine the effect of PG-1 in a murine burn model after bacterial infection with a silver sulfadiazine resistant strain of *Pseudomonas aeruginosa*. Depilated male Sprague-Dawley rats received a 10% total body surface area partial-thickness burn by immersion in a 60 °C water bath for 15 s followed by wound seeding with 10^5 CFU *P. aeruginosa*. Occlusive dressings were applied to prevent cross contamination. After 48h the rats were randomized into four groups: 1) 20μg recombinant PG-1, 2) 0.01% acetic acid (carrier), 3) 500ng Gentamycin, and 4) no treatment. Treatment was given by intradermal injection with a 30G needle. The following day, the wound tissues were harvested aseptically, weighed, homogenized and plated on *Pseudomonas* isolation agar after appropriate serial dilutions. Bacterial plates were then incubated for 18h and the numbers of bacterial colonies were counted in a blinded fashion. Bacterial quantitative wound cultures revealed significant bacterial killing in the PG-1 group as compared to the negative control group. This study shows that PG-1 may be used as a potential alternative or adjunct treatment to standard topical agents in infected burn wounds. As a small protein it is also a potential candidate for gene therapy.

Funding: NIH (HL-03803, DK-53296, AI-01030, GM-54911) and a VA Merit award

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Burn-induced T cell suppression is prevented after neutrophil depletion in burn-injured rats.

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Previous studies from our laboratory have shown an up regulation of neutrophil activity and suppression of T cell proliferation following burn injury in rats. We hypothesized that the activated neutrophils play a role in the suppression of T cell responses. The present study evaluated the role of neutrophil in affecting T cell proliferative response in Peyer's patches (PP) and mesenteric lymph nodes (MLN) of burn injured rats. These studies were performed in the rat model of full thickness skin scald over 25% TBSA. The experimental animals were pretreated with either phosphate-buffered saline (Burn+PBS) or PMN antibody iv (Burn+PMN ab) prior to burn injury, and sacrificed 48 hours later. Anti PMN ab is a polyclonal rabbit anti-rat PMN antiserum diluted in 1 ml of PBS. A single injection of 150 μg of anti PMN ab was sufficient for maintaining neutrophil depletion for 48 hours after burn injury.

Proliferative response (DPM X 10^{-3})			
Organ	Control	Burn+PBS	Burn+PMN antibody
PP	62.58±4.78	5.78±1.57*	53.92±3.27
MLN	188 ± 22	127 ± 5*	196 ± 36

P<0.001, Burn+PBS vs. Burn+PMN ab or Control

These results indicate that the diminished T cell proliferative response in PP's and MLN following burn injury is prevented by depleting neutrophils in burn injured rats. Our data support that neutrophils can cause

suppression of mucosal T cell proliferation which occurs with burn injury.
(Support from NIH GM 53235 and GM 56865).

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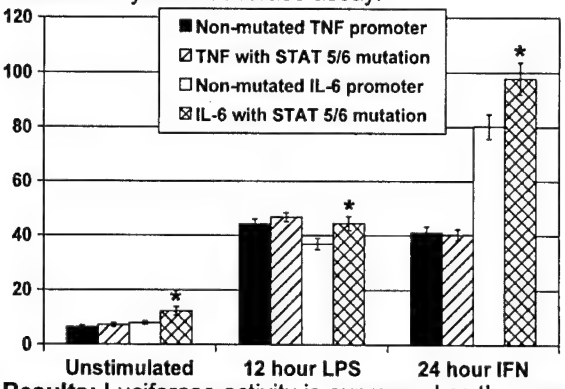
STAT 5/6 PROTEIN AND CYTOKINE EXPRESSION.
V. Chappell*, L. LaGrone*, W. Mileski , UTMB Medical Branch, Galveston, TX 77555.

Signal transducer and activator of transcription proteins (STATs) mediate activation of several cytokine genes. We identified a centrally-located STAT 5/6 binding site within the promoter region for both TNFα and IL-6 and hypothesize that alterations in this site would affect expression. **Methods:** The 1.9Kb TNF and 1.3Kb IL-6 promoters were inserted in separate luciferase reporter vectors and the binding site for STAT 5/6 mutated using site directed mutagenesis. Murine macrophages were transfected with the resultant plasmids, then incubated with and without LPS (1.0μg/ml) and IFN-γ (100U/ml). Gene expression was measured by dual luciferase assay.



Results: Luciferase activity is expressed as the average relative light intensity (± SEM) * indicates $p<0.05$ vs. non-mutated IL-6, t-test. Mutation of the STAT 5/6 binding site had no effect on TNF expression. Mutation of the STAT 5/6 binding site on the IL-6 promoter increased constitutive expression as well as LPS- and IFN-inducible expression. **Conclusions:** Mutation of the STAT 5/6 binding site increases IL-6 gene expression, while it has no affect on TNF gene expression.

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THE INFLAMMATORY RESPONSE IN SEVERELY INJURED PATIENTS FOLLOWING SMALL VOLUME RESUSCITATION
UC Liener, AK Bauer*, M Helm#, L Kinzl*, UB Brückner‡ F Gebhard: Dept Traumatol & Div Surg Res‡, University of Ulm and German Army Hospital Ulm#, 89075 Ulm Germany

Small volume resuscitation (SV) restores blood pressure and organ perfusion to preshock values in hypovolemic shock. The influence of SV on the inflammatory reaction is unclear and not reported so far. In a prospective randomized trial we analyzed possible alterations in macrophage response (MIP-1ß), PMN elastase release and expression of soluble intracellular adhesion molecules (sICAM) in the earliest preclinical period following trauma. **Methods:** Upon approval of the IRB/IEC, 41 patients (pts) with multiple injuries (mean ISS 34) were enrolled. A subset of 14 pts with severest trauma (ISS>32) who received either standard resuscitation, i.e. starch & crystalloids (C= control) or hyperosmolar starch (SV= small volume) was analyzed. The first blood sample was obtained at the scene of the accident prior to cardiopulmonary resuscitation, when appropriate. Then, blood samples were collected hourly for 24 hrs, then at day 5 and 10. **Results:** There were 5 pts (median ISS 41; 33-75) in group C and 9 pts in group SV (median ISS 41; 34-75). During the observation period 9 pts died. All measured variables promptly increased in all pts within 2 hrs after the injury and showed a comparable time course. There was a two- to threefold increase of MIP-1ß and sICAM during the observation period in group SV compared to group C. SV pts also showed a more pronounced (twofold) elastase release then group C pts. **Discussion:** Our preliminary results indicate that the inflammatory response in pts receiving small volume resuscitation is increased compared to patients infused with standard therapy. This pilot study is to be continued in order to further scrutinize these findings and to elucidate a possible effect on the outcome.

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INTRACELLULAR MECHANISM OF INTESTINAL T CELL SUPPRESSION FOLLOWING BURN INJURY.
M. A. Choudhry, S. Namak*, X. Ren*, S. Khan* and M. M. Sayeed, Trauma/Critical Care Research Labs, Loyola University Chicago Med. Sch., Maywood, IL 60153

Previous studies from our laboratory have suggested that burn suppresses intestinal T cell function via down-regulation of T cell receptor mediated $P59^{fyn}$ activation. Since the activation of both src kinases ($P56^{lck}$ and $P59^{fyn}$) and Zap-70 is needed for subsequent signaling cascade to lead to cell activation, the present study examined the effect of burn on $P56^{lck}$ and Zap-70 to delineate whether burn mediated inhibition of T cell functions is due to $P59^{fyn}$ attenuation selectively or $P56^{lck}$ and Zap-70 are also affected. Rats (~250 g) were subjected to 25% total body surface area. T cell were isolated from Peyer's patches (PP) and mesenteric lymph nodes (MLN) on day 3 post burn injury. *In vtro* kinase assay was used to measure $P56^{lck}$ autophosphorylation (auto) and kinases activity (enolase). Immunoblotting was used to measure phosphorylation of Zap-70. The results are:

Experiment	Densitometric Units		
	$P56^{lck}$		Zap-70
PP	auto	enolase	auto
Control	1±0.1	1±0.1	1± 0.2
Burn	0.5+0.2	0.4±0.1	0.6+0.2
MLN			
Control	1±0.1	1±0.1	1± 0.2
Burn	0.6+0.2	0.5±0.1	0.6+0.2

These results suggest a suppression of both $P56^{lck}$ and Zap-70 in both PP and MLN T cells of burn rats compared to control. Such a suppression of $P56^{lck}$ and Zap-70 along with $P59^{fyn}$ may disrupt the signal from T cell receptor to the down-stream cascade, which may arrest T cells activation and thus impair cell function. (Support: NIH grants GM53235 and GM56865)

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Calpain inhibitor I Reduces the Colon Injury Caused by Dinitrobenzene Sulfonic Acid in the Rat

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[1] Institute of Pharmacology, [3]Department of Biomorphology, [4]Institute of General Surgery, University of Messina, Italy; [2]The William Harvey Research Institute, London United Kingdom.

The aim of the present study was to examine the effects of calpain inhibitor I in rats subjected to experimental colitis. The aim of the present study was to examine the effects of calpain inhibitor I in rats subjected to experimental colitis. Colitis was induced in rats by intra-colonic instillation of dinitrobenzene sulfonic acid (DNBS). Rats experienced hemorrhagic diarrhoea and weight loss. At 4 days after administration of DNAB, the mucosa of the colon exhibited large areas of necrosis. Neutrophil infiltration (determined by histology as well as an increase in myeloperoxidase activity in the mucosa) was associated with up-regulation of ICAM-1 and P-as well as high tissue levels of malondialdehyde. Immunohistochemistry for nitrotyrosine and poly (ADP-ribose) polymerase (PARP) showed an intense staining in the inflamed colon. Staining of sections of colon obtained from DNBS-treated rats with an anti-COX-2 antibody showed a diffuse staining of the inflamed tissue. Furthermore, expression of inducible nitric oxide synthase (iNOS) was found mainly in macrophages located within the inflamed colon of DNBS-treated rats. Calpain inhibitor I (5 mg/kg daily i.p.) significantly reduced the degree of hemorrhagic diarrhoea and weight loss caused by administration of DNBS. Calpain inhibitor I also caused a substantial reduction of (i) the degree of colonic injury, (ii) the rise in MPO activity (mucosa), (iii) the increase in the tissue levels of malondialdehyde, (iv) the increase in staining (immunohistochemistry) for nitrotyrosine and PARP, as well as (v) the upregulation of ICAM-1 and P-selectin caused by DNBS in the colon. Thus, calpain inhibitor I reduces the degree of colitis caused by DNBS. We propose that calpain inhibitor I may be useful in the treatment of inflammatory bowel disease.

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PROTEIN TYROSINE KINASE LYN IS UP-REGULATED IN NEUTROPHILS OF BURN-INJURED RATS.

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Hyperactivation of neutrophils in early stages after burn injury plays a role in oxidative injury in endothelial and parenchymal tissues in a variety of organs of the injured host. Such neutrophil hyperactivation may be accompanied by up-regulation of selective neutrophil signaling pathways triggered by a selective group of burn-injury related inflammatory mediators. Previous studies have assessed burn animal derived neutrophil signaling pathways that are dependent on Ca^{2+}. In this study we assessed neutrophil signaling which is initiated via Src kinase Lyn, and leads to neutrophil oxidant production in the absence of a generated Ca^{2+} signal. Neutrophils harvested from sham and burn rats (25% TBSA, day 1 post-burn), were stimulated with fmlp for 3 minutes and lysed. Cells lysates were immunoprecipitated with anti-Lyn antibodies. The immunoprecipitated Lyn from sham and burn animals was then assessed for autophosphorylation and kinase activity (assessed by the ability to phosphorylate substrate enzyme enolase) using ^{32}P-γATP and *in vitro* kinase assay. The results show an up-regulation of Lyn kinase activity in neutrophils on day 1 post-burn as compared to sham controls. In conclusion, neutrophil hyperactivation in early stages of burn injury is dependent also on an up-regulation of the Ca^{2+}-independent signaling pathway involving Lyn. (Support: NIH grants GM53235 and GM56865)

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CROSS TOLERANCE BETWEEN LPS AND TNFα OR IL-1β; EFFECT ON CELLULAR SIGNALING.

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LPS tolerance induces cross-tolerance to TNFα and vice versa. Since LPS tolerance alters proximal signal transduction events, it was hypothesized that impaired signaling through common pathways is a mechanism for heterologous-tolerance. Because recent studies indicate similarities in IL-1β and LPS signaling, we also hypothesized that cross-tolerance between LPS and IL-1β occurs. Human THP-1 cells were rendered tolerant by pretreatment with LPS, TNFα or IL-1β for 18 hrs, and were subsequently restimulated with either LPS, TNFα or IL-1β for 40 min. Signaling was quantitated from inhibitor κB (IκBα) degradation and nuclear translocation of nuclear factor κB (NF-κB) p65 and p50 hetero/homodimers complexes. In control cells (non pretreated), LPS, TNFα or IL-1β induced IκBα degradation (>90%, n=3) and nuclear translocation of NF-κB p65/p50 heterodimers (Fig. 1).

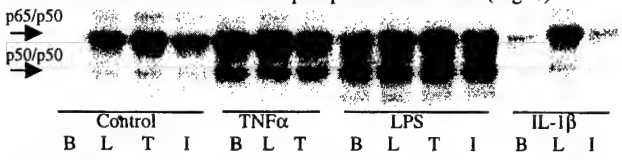

Fig.1. Representative EMSA (n=3) showing nuclear translocation of NF-κB in Control and TNF(10ng/ml), LPS(1ug/ml) or IL1(100ng/ml) pretreated THP-1 cells and restimulated with LPS (L-10ug/ml), TNF (T-10ng/ml) or IL-1 (I-100ng/ml); (NF-κB-DNA binding are shown by arrows).

In LPS and TNFα tolerant cells a different pattern in NF-κB composition was observed, with an increase in p50/p50 homodimers, which remained unaltered following stimulation. In contrast, IL-1β tolerant cells showed no change in basal NF-κB translocation. LPS tolerance induced cross-tolerance to subsequent TNFα or IL-1β stimulation as determinated by inhibited IκBα degradation and increased nuclear NF-κB p50 subunits. TNFα tolerance induced similar cross-tolerance to LPS induced signaling. However, IL-1β tolerance did not produce cross-tolerance to LPS. These findings suggest common signaling pathways inducing cross-tolerance for LPS and TNFα, which are distinct from IL-1β signaling events. Supported in part by NIHGM27673 and MUSC Postdoctoral Fellowship.

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p38 BUT NOT ERK KINASE UP-REGULATION IS DEPENDENT ON Ca^{2+} SIGNALING IN BURN INJURED RAT NEUTROPHILS. M. J. Flanagin*, M. A. Choudhry, N. Fazal*, M. M. Sayeed Trauma/Critical Care Research Labs, Loyola University Medical Center, Maywood, IL 60153

The hyperactivation of circulating neutrophils in rats in early stages after burn injury is accompanied by an up-regulation of Ca^{2+} signaling triggered by ligands which activate receptors made up of seven membrane spanning domains. The same receptors as well as TNFR and GM-CSFR also stimulate neutrophils via Ca^{2+}-signaling independent pathways which include activation of MAPKs. We have evaluated whether the Ca^{2+} signaling up-regulation in burn injured rats is accompanied by activation of MAPKs Erk1/2, and p38, and whether any of these two families of MAPKs are dependent on the Ca^{2+} signal generation in the burn inflammatory conditions. Blood neutrophils were isolated from sham and burn rats (25% TBSA skin scald), day 1 post burn, and analyzed for their ability to mobilize cell Ca^{2+} and activate Erk and p38 kinases in response to fMLP (1μM). Ca^{2+} mobilization was measured using Fura-2 microfluorometry and kinases were evaluated by assessing phosphorylated Erk and p38 proteins using Western blots. A group of rats were treated with diltiazem (DZ) (2 mg/kg) at the time of the burn procedure. The treatment with DZ prevented the up-regulation of neutrophil Ca^{2+} mobilization with burn injury. The MAP kinase data (blot analyses in densitometric units) indicated burn-induced up-regulation of both Erk and p38 kinases.

Experiment	Sham	Burn	Burn + DZ
Erk 1/2	85±2	160±24	144±35
p38	23±5	58±5	40±6

The treatment of burn rats with DZ prevented the up-regulation of p38 ($p < 0.05$) but not Erk activation. These results suggest that Ca^{2+} signaling may play a role in the activation of p38 but not Erk in neutrophil of burn injured rats neutrophils. (Support: NIH grants GM53235 and GM56865)

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ENHANCED VESSEL RESPONSES TO PHENYLEPHRINE (PE) AND ACETYLCHOLINE (ACH) OVER TIME (2 HOURS) IN MICE. P. D. Harris, J. Hu*, T. Kawabe, R. N. Garrison. Depts Physiology & Surgery & Center Applied Microcirculatory Res, Univ. Louisville & Veterans Administration, Louisville, KY, 40292.

Increased wall stretch (preload, PL) increases vessel response to receptor-mediated PE-induced contractions and to Nitric-Oxide (NO)-mediated ACH-induced relaxations in mouse aortic rings. Our present study asked: Does this preload effect change over time for either agonist? Thoracic aortic rings (1.5mm) from mice (n=8) were put on 1 of 7 different PL (100-700mg); pretreated with 1uM ACH and 1uM PE for 10mins; and washed for 25mins. Rings were contracted with 6 doses of PE (0.01-3.0uM) and relaxed with ACH (3.0uM). This cumulative PE dose-response curve + ACH was done twice over 2 hours (HR) in each ring. Max change in force to PE and ACH, and in PE reactivity (pD2=-log dose for 50% response) were analyzed by ANOVA. PE Max was higher in HR2 (189mg at 100mg PL to 473mg at 700mg PL, SEM 25mg) than in HR1 (115mg at 100mg PL to 394mg at 700mg PL, SEM 25mg) at all preloads. PE reactivity (pD2) was higher in HR2 (6.950 at 100mg PL to 7.079 at 700mg PL, SEM 0.0372) than in HR1 (6.897 at 100mg PL to 6.898 at 700mg PL, SEM 0.0372) mostly at preloads above 400mg. ACH Max relaxation was higher in HR2 (56.0% at 100mg PL to 66.1% at 500mg PL to 57.0% at 700mg PL, SEM 1.5%) than in HR1 (50.6% at 100mg PL to 57.6% at 500mg PL to 47.9% at 700mg PL, SEM 1.5%) at all preloads. A post-receptor preload-dependent mechanism enhances receptor-operated PE-induced calcium-channel-mediated contraction and this preload mechanism is enhanced at all preloads within two hours after PE exposure. This enhancement of PE-induced contractions does not involve reduced endothelial NO-release since ACH-induced relaxation is also enhanced within two hours at all preloads.(Funded:CAMR, VA Merit, US Dept Defense)

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OPTIMAL PRELOAD TO GIVE MAXIMUM VESSEL RESPONSES TO PHENYLEPHRINE (PE), POTASSIUM (K), AND ACETYLCHOLINE (ACH) IN MICE. J. Hu*, P. D. Harris, T. Kawabe, R. N. Garrison. Depts Physiology & Surgery & Center Applied Microcirculatory Res, Univ. Louisville & Veterans Administration, Louisville KY 40292.

Increased wall stretch (preload, PL) increases vessel response to receptor-mediated PE-induced contractions and to voltage-mediated K-induced contractions, but decreases response to Nitric-Oxide (NO)-mediated ACH-induced relaxation of rat aortic rings. Our study determined if there is an optimal vessel wall PL which gives maximal contractions to PE and K while maintaining response to NO in mouse aortic rings. Thoracic aortic rings (1.5mm) from mice (n=8) were put on 7 PL (100-700mg); pretreated with 1uM ACH and 1uM PE for 10mins; and washed for 25mins. Rings were contracted with 6 doses of PE (0.01-3.0uM), relaxed with ACH (3.0uM), and contracted with 80mM Potassium. Max change in force to PE, K, and ACH, and in PE reactivity (pD2=-log dose for 50% response) were analyzed by ANOVA. PE Max increased from 152mg at 100mg PL to a plateau of 374mg at 400 to 600mg PL and then increased to 433mg at 700mg PL (SEM 8mg). PE reactivity (pD2) was unchanged (6.804 to 6.899; SEM 0.053) over 100 to 700 mg PL. K Max increased from 416mg at 100mg PL to a plateau of 744mg at 500 to 700mg. ACH Max increased from 53.3% relaxation at 100mg PL to a peak of 61.8% at 500mg PL and then declined to 52.5% relaxation at 700mg PL. A post-receptor (no PL effect on PE pD2) preload-dependent mechanism enhances receptor-operated PE-induced and voltage-operated K-induced calcium-channel-mediated contractions from 100 to 700 mg PL in mice. This preload mechanism appears to enhance PE and K induced contractions by reduced endothelial NO-release above but not below 500mg PL, since increased PL reduces ACH-induced relaxation only above 500mg PL. (Funded: CAMR, VA Merit, US Dept Defense)

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INTERACTIONS BETWEEN LIPID AND PEPTIDE G-PROTEIN COUPLED RECEPTORS IN RAT PMN MODEL HUMAN INFLAMMATION. CJ Hauser, Z Fekete*, JM Adams*, CA Adams*, RM Forsythe*, DZ Xu, JT Sambol*, Q Lu*, D Anjaria*, DH Livingston and EA Deitch. **UMDNJ**, Newark, NJ 07103.

BACKGROUND: PMN responses to G-protein coupled (GPC) chemoattractants are crucial to systemic inflammatory responses but species differences may make rodent models of trauma, shock and sepsis inappropriate for the study of human disease. We validated using rat PMN as a model for GPC signaling in humans by comparing the cross-regulation of chemokine and lipid receptors using $[Ca^{2+}]_i$ mobilization as a marker for responses.

METHODS: Rat or human (**Hu**) PMN were isolated by specially adapted one-stage separations. Cells were fura-loaded and assayed by fluorimetry. Basal $[Ca^{2+}]_i$ and responses of **Hu** PMN to GRO-α (**G**), IL-8 (**8**) and PAF (**P**) were compared to responses of rat PMN to the putative equivalents rat GRO (**RG**), MIP-2 (**M**) or PAF (**P**) with each agonist given in an EC50 dose as an initial and a primed stimulus. (**X→Y** indicates the response to **Y** after **X**)

RESULTS: * $p < 0.05$

	Basal	Peak Ca^{2+} response to EC50 dose (nM/L)			
		8	G→8	P→8	8→G
Hu	43±2	199±9	238±13*	220±8*	no activity
		M	RG→M	P→M	M→RG
Rat	50±2	95±7	127±14*	148±1*	no activity

Conclusions: Circulating rat PMN show similar but slightly higher basal and slightly lower stimulated $[Ca^{2+}]_i$ than human PMN. EC50 agonist doses are also lower. In contrast, the priming and suppressive interactions between lipid and peptide agonists were qualitatively and proportional very alike in the two species. RG and MIP show the same relationship in rats that GRO-α and IL-8 do in man. Rat PMN may prove a useful model for studying PMN responses to GPC agonists in trauma, shock and sepsis.

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ACTIVATION OF $P2X_7$ AND CA^{2+} FLUX IN GH3 CELLS. JW LEE, HS Chung*, KS Park*, SK Cha* and ID Kong*. Dept. of Physiology Yonsei Univ. Wonju College of Medicine, Wonju Korea.

Extracellular ATP plays a role in Ca^{2+} signaling and hormone secretion in the endocrine system. However, it has not yet been elucidate which subtypes of purinergic receptor are expressed in pituitary cells (GH3) and which mechanisms are involved. A fluorometric, an electrophysiological and a reverse transcriptase- polymerase chain reaction (RT-PCR) techniques were conducted in GH3 cell line. ATP and BzATP increased $[Ca^{2+}]_i$, with EC_{50} values of 651 μM and 18 μM, respectively. The responses were dependent upon the extracellualr Ca^{2+} concentration. Preincubation with oxidized ATP (oATP) nearly abolished the ATP- and BzATP-induced $[Ca^{2+}]_i$ increases. Both ATP and BzATP induced depolarization with EC_{50} values of 1 mM and 31 μM, respectively. The rank order of agonist potency for $[Ca^{2+}]_i$ and depolarization responses was BzATP >> ATP > 2-MeSATP and other purine derivatives such as ADP, AMP, adenosine were ineffective. Neither UTP nor α, β-methylene ATP showed any effect. BzATP evoked non-desensitizing inward currents, which reversed at ~ 0 mV. $P2X_7$ mRNA on GH3 cells was identified by using RT-PCR. These results suggest that the GH3 cells have an endogenous $P2X_7$ receptor and purinergic stimulation may play a potential role in neuroendorine modulation via changes in intracellular Ca^{2+} concentration and ionic currents.

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EXPRESSION OF HEAT SHOCK PROTEINS IN RESPONSE TO ALTERED BLOOD FLOW IN VIVO. S.Mondy, L.Knoepp*, C.Brophy*, Med. Col. of GA and Augusta VAMC, Augusta, GA 30912

Heat shock proteins (HSP) are involved in regulation of vascular tone and may modulate the arterial response in pathologic states such as hypertension, shock, and sepsis syndromes. We investigated the hypothesis that expression of HSP by vascular smooth muscle cells (VSMC) is altered in response to changes in blood flow. In a well-characterized rat model of altered blood flow (n = 8), the right internal and external carotid artery branches were ligated to decrease flow in the right common carotid artery (CCA). The left side was sham-operated only. This results in a 94% reduction in flow in the right CCA (Reduced Flow, RF) and a compensatory 50% increase in flow in the left CCA (Increased Flow, IF). After two weeks, immunohistochemical staining was performed on fixed cross sections, and western blot analysis was performed on common carotid smooth muscle using antibodies specific for HSP20, HSP27 and phosphorylated HSP27 (HSP27-P). Protein expression was determined by densitometric analysis of the western blots. Results are reported as a ratio of RF to IF. Comparison was made to baseline (RF/IF = 1) using ANOVA, and a $p<0.05$ was considered significant. RESULTS: HSP27 and HSP27-P increased in RF versus IF arteries (1.70 ± 0.34 and 2.37 ± 0.52 respectively, $p < 0.05$). HSP20 protein expression decreased in RF versus IF arteries (0.53 ± 0.10, $p < 0.05$). These findings were qualitatively confirmed on examination by light microscopy. These data suggest that blood vessels respond to altered flow with changes in small HSP expression in VSMC. Since HSP20 modulates vasorelaxation and HSP27 modulates vasoconstriction, alterations in HSP expression may modulate dynamic caliber changes in vessels in response to flow. These proteins may provide targets for therapeutic intervention in pathologic states characterized by deranged vascular tone.

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ACTIVATION OF ERK-1 AND ERK-2 IN T CELL FOLLOWING BURN. X. Ren*, S. Namak*, S. Khan*, M. A. Choudhry, and M. M. Sayeed, Trauma/Critical Care Research Labs, Loyola University Chicago Medical School, Maywood, IL

The mitogen activated protein kinases (MAPK) are components of signal transduction pathways which respond to a variety of extracellular stimuli. Upon activation, MAPK serve to relay, amplify, and integrate diverse signals, thus allowing the cell to coordinate a physiological response. More than 10 isoforms of MAPK are classified according to their differential response to various agonists. In the present study, we examined the activation of Erk-1 (42 kDa) and Erk-2 (44 kDa) in T cells obtained from Peyer's patches (PP) and mesenteric lymph nodes (MLN) of control and burn rats. Both Erk-1 and Erk-2 are activated by phosphorylation on tyrosine and treonine residues. Immunoblotting was used to measure the tyrosine phosphorylation of both Erk-1 and Erk-2. The results are:

Experiment	Densitometric Units	
	Phosphorylation state	
PP	Erk-1	Erk-2
Control	1±0.12	1±0.06
Burn	0.6±0.15	0.75±0.08
MLN		
Control	1±0.1	1±0.1
Burn	0.58±0.1	0.64±0.08

These results suggest a greater suppression of Erk-1 than Erk-2 in PP T cells of burn rats compared to controls. In contrast burn suppressed both Erk-1 and Erk-2 in MLN T cells equally. Suppression of Erk-1 and Erk-2 may impair subsequent signal transdcution and thus block T cell function following burn injury. (Support: NIH grants GM53235 and GM56865)

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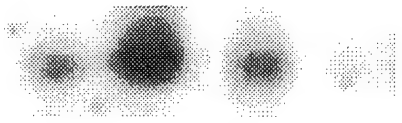
ALTERATIONS IN GLUCOSE-6-PHOPHATASE GENE EXPRESSION IN LATE HEMORRHAGE/ RESUSITATION. Tunc Aksehirli, Tariq Khan, Dorrie-Sue Barrington, Subir Maitra, Dept of Emergency Medicine, SUNY at Stony Brook, NY 11794

Previous studies have shown an increase in Glucose-6-Phosphatase expression 30 min after hemorrhage/ resuscitation. In the present study, we have determined its abundance in late hemorrhage/resuscitation. Rats were anesthetized and subjected to hemorrhagic shock for 30 min at a mean arterial pressure of 40mmHg and then resuscitated with Lactated Ringer's (HS/LR) to a mean arterial pressure of 90mmHg for 30 min. The animals were studied at four different time periods: Control; 30 min following HS/LR; 5 hrs following HS/LR; and 24 hrs following HS/LR. Liver samples were freeze-clamped at the end of each experiment for Northern Blot analysis of Glu-6-Pase. Northern Blot analysis revealed an abundance of Glu-6-Pase. There was a 4-fold increase at HS/LR (B) compared to Control (A). After 5 hrs of resuscitation (C) intensity returned to control values, and expression after 24 hrs (D) decreased to almost undetectable levels.



A B C D

Our data indicate that *in vivo* acute up-regulation and subsequent down-regulation of Glu-6-Pase gene expression are associated with hyperglycemic and hypoglycemic phase of HS/LR. These results support the important regulatory role of Glu-6-Pase for hepatic glucose output during progression of HS/LR. (Supported by NIH GM 58047 and GM 52025)

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TESTOSTERONE RECEPTOR BLOCKADE FOLLOWING TRAUMA-HEMORRHAGE RESTORES ORGAN BLOOD FLOW AND IMPROVES TISSUE OXYGEN CONSUMPTION. Z.F. Ba*, P. Wang, D.J. Koo*, and I.H. Chaudry, Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Although studies have shown that testosterone receptor blockade with flutamide restores the depressed immune function in male animals following trauma and hemorrhagic shock, it remains unknown whether the salutary effects of this agent are due to improved organ blood flow (BF) and tissue oxygen consumption (VO_2) under such conditions. To study this, male rats (275-325g) underwent laparotomy and were then bled to and maintained at an arterial BP of 40 mmHg until 40% shed blood volume was returned in the form of Ringer's lactate (RL). They were then resuscitated with 4X the volume of shed blood with RL over 60 min. Flutamide (25 mg/kg) or an equivalent volume of the vehicle propanediol was injected s.c. 15 min before the end of resuscitation. At 24 h post-resuscitation, organ BF (ml/min/100g BW) was determined using ^{85}Sr microspheres, and blood samples (0.15 ml each) were collected from the femoral artery, portal, hepatic and renal veins to measure their oxygen content using a hemoximeter. The VO_2 values (ml/min/100g BW) were then calculated.

	Sham	Hem	Hem+Flu
Hepatic BF	160.3±8.2	99.6±9.9*	137.7±4.4#
Intestinal BF	112.7±6.4	75.3±5.9*	107.4±4.0#
Renal BF	496±33	341±39*	389±21
Hepatic VO_2	14.48±1.72	2.48±0.10*	3.70±0.30*#
Intestinal VO_2	10.04±1.00	2.29±0.26*	4.88±0.52*#
Renal VO_2	33.83±7.24	8.04±1.25*	12.48±1.27*#

(Hem + Flu; hemorrhage with flutamide treatment. Data are presented as mean ± SE, n=6/group, and compared by ANOVA and Tukey's Test: *P < 0.05 vs. Sham; #P < 0.05 vs. Hem)

The results indicate that administration of flutamide after hemorrhage improved tissue perfusion in the liver and gut and increased VO_2 in all the tested organs. In addition, O_2 extraction ratio increased significantly following flutamide administration, as compared to vehicle-treated hemorrhaged animals and even sham animals. The improved BF and VO_2 following the administration of flutamide appears to be responsible for the beneficial effects of this androgen receptor antagonist on immune and other cell and organ functions in males following trauma and hemorrhagic shock (NIH grant R37 GM 39519).

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MORPHOLOGIC CHANGES OF RBCs DURING HEMORRHAGIC SHOCK REPLICATE CHANGES OF AGING. T.Berezina*, S.Zaets*, V.Kozhura*, I.Novoderzhkina*, A.Kirsanova*, E.Deitch and G.Machiedo.UMD- NJ Medical School, Newark, NJ 07103 and Institute General Reanimatology, Moscow, Russia 103031

It is known that blood loss leads to the increase of the number of prehemolytic forms of red blood cells (RBCs). However, the changes in morphology at different stages of hemorrhagic shock have not been studied. The aim of this work was to study the morphological parameters of RBCs during massive blood loss. **Methods**: The study was performed on 15 adult inbred narcotized dogs. The blood samples were taken before the blood loss, when the arterial pressure reached 40 mm Hg (3.0±1.9 min), and afterwards, at the arterial pressure level of 20 mm Hg (9.3±3.5 min). The volume of blood lost averaged 33.6±8.9 ml/kg and 55.1±6.9 ml/kg, respectively. The evaluation of morphological parameters of RBCs was performed by means of computerized light microscopic morphomtery, and scanning electron microscopy. **Results**: At the initial stage of blood loss the number of "young-appearing" RBCs with large visible surface area (40-50 mkm2) increased from 17.7±3.1% to 26.6±3.5% ($p<0.05$). The number of "old-appearing" RBCs with small visible surface area (20-30 mkm2) significantly decreased from 5.3±2.7% to 2.7±2.3% ($p<0.01$). At the stage of decompensated blood loss, an opposite phenomenon was observed. The number of "old-appearing" RBCs increased to 8.2±1.1% ($p<0.01$), whereas the number of "young-appearing" RBCs progressively decreased to 12.3±4.2% ($p<0.01$). The change in visible surface area of RBCs was accompanied by significant alterations of their shape. The percentage of abnormal shaped RBCs increased from 8.9±1.1% before the blood loss to 36.4±5.8% at the stage of decompensated hemorragic shock ($p<0.01$). **Conclusions**: During hemorrhagic shock, the shape changes and changes in surface area are similar to those seen in aging. This may be due to the effects of oxidative stress in both conditions.

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THE EFFECT OF ENALAPRILAT ON PORTAL VEIN AND SUPERIOR MESENTERIC ARTERY FLOW. A. Cárdenas*, P. Wall, L. Henderson*, C. Buising*, T. Rickers*, F. Raymond*, R. Vincent*, K. Daniels*, S. Bell*, L. Owens*, A Chendrahsehkar, D. Moorman*, G. Timberlake. Surg Ed & Trauma, IA Methodist Med Ctr, & Drake U, Des Moines, IA 50311.

Enalaprilat administration during resuscitation may be useful for improving splanchnic blood flow. **Methods:** Ten dogs were anesthetized, instrumented, and bled to a MAP of 40-45mmHg, then 30-35mmHg for periods of 30min. The dogs were then resuscitated to MAP 40-45mmHg for 30min. At this point 5 of the dogs were given a constant rate infusion (CRI) of enalaprilat (0.02mg/kg/h), and the other 5 received saline (120min each grp). Blood flow was measured in the portal vein (PV) and the superior mesenteric artery (SMA) using a Transonics® doppler ultrasound system. **Results:** Flow decreased in both groups during hemorrhage. SMA flow decreased 44% and 34% while PV decreased 40% and 80% in enalaprilat and saline dogs, respectively. No difference in flow before CRI.

	Start CRI		*120 minutes after CRI*	
	SMA (ml/min±SEM)	**PV** (L/min ±SEM)	**SMA** (ml/min ±SEM)	**PV** (L/min ±SEM)
Enalaprilat	46.8 ± 5.3	0.22 ± 0.12	88.6 ± 41.3	0.38 ± 0.09
Saline	68.0 ± 5.3	0.14 ± 0.03	67.0 ± 5.0	0.17 ± 0.03
P-Values	0.29	0.52	0.043	0.066

During enalaprilat SMA flow increased by 90% and PV flow increased by 74%. Saline dogs showed a 1.5% SMA decrease and a 21% PV increase in flow during resuscitation. **Conclusions:** Enalaprilat during resuscitation improved splanchnic blood flow. A constant rate infusion of enalaprilat in trauma patients might be useful for increasing splanchnic blood flow. (Support: Drake U, Pfizer, VA Central IA Health Care Sys, DM Research & Ed Corp, Arrow Int'l, Diametrics, IA Space Grant Consortium)

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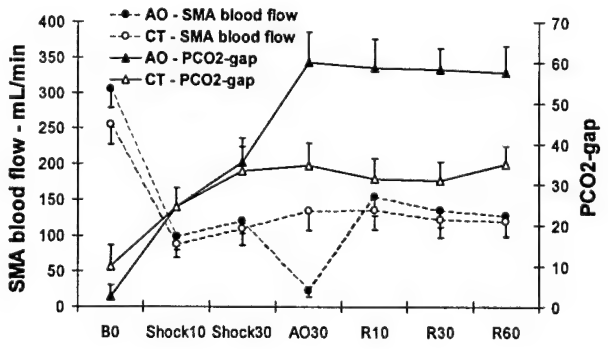
SPLANCHNIC BLOOD FLOW, OXYGEN METABOLISM AND PCO_2-GAP AFTER AORTIC OCCLUSION DURING HEMORRHAGIC SHOCK. RJ Cruz Junior*, LF Poli de Figueiredo, JLM Braz*, C Lagoa*, M Rocha e Silva. Research Division, Heart Institute – InCor, Univ. of São Paulo Medical School, SP 05403-000, Brazil.

Aortic occlusion (AO), has been suggested for the initial treatment of severe uncontrolled hemorrhagic shock. Our objective is determine the impact of AO on splanchnic blood flow and gastric tonometry. Methods. Fourteen dogs (17± 1.8 Kg) were bled to a mean arterial pressure (MAP) of 40mmHg. After 30 min, animals were randomized to: AO (transfemoral aortic occlusion at T9 level for 30 min, n=7) or CT (controls, no occlusion, n=7). Mesenteric blood flow (MBF, ultrasonic flowprobe), gastric mucosal perfusion (gastric tonometry) and intestinal oxygen extraction ratio (O_2ERi) were evaluated for 120 min. Results. Hemorrhage induced a significant reduction on MAP, MBF and increases in PCO_2-gap and O_2ERi. Aortic occlusion significantly improved MAP, and further increased PCO_2-gap and O_2Eri, with a decreased MBF. After reperfusion MBF, MAP and O_2ERi returned to pre-occlusion values, although PCO_2-gap remained higher in AO group. AO produces severe impairment in mucosal blood flow in spite of partial restoration of MBF during the reperfusion period.



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EFFECT OF RESUSCITATION FLUID RATES ON OUTCOME. D. Davis*, P.Wall, A. Cárdenas*, T. Mattson*, A. Larkin*, L. Wittkopf*, F. Raymond*, D. Moorman*, G. Timberlake. Surg Ed & Trauma, IA Methodist Med Ctr, Des Moines, IA 50309.

Rapid resuscitation with lactated Ringer's has been suggested to contribute to immunosuppression post-hemorrhage.[1] We investigated resuscitation fluid rate effects on survival. **Methods:** 30 Wistar-Furth rats were anesthetized; instrumented; hemorrhaged (MAP=35-40mmHg >60min until MAP<30mmHg for >10min or <25mmHg for >1min, or 120min elapsed); and resuscitated (R) for 3hr with room temperature lactated Ringer's (LR) intermittently to keep MAP=75-80mmHg. When running, the LRS rates were 15ml/hr (50.3ml/kg/hr), 30ml/hr (103.9ml/kg/hr), and 60ml/hr (210.9ml/kg/hr). Rats were euthanized at 24hr. **Results:**

	#alive start R	#alive 24hr	ΔBE 2hr R	ΔT 3hr R	3hr LR ml/kg
15ml/hr	6	3	5.0±1.0	-0.7±.3	44±8
30ml/hr	7	2	5.4±0.8	0.5±0.4	47±3
60ml/hr	8	5	7.1±1.1	1.7±0.6	89±17

(Average ± SEM.) Rats required LR at their maximum rates for approximately the first 15, 10, and 5 min of resuscitation (15ml/kg, 30ml/kg, & 60ml/kg respectively). The slopes of LR received lines in the 15 and 30 ml/hr rats were not significantly different at any time. The slope of the LR received line was greater in the 60 ml/hr rats throughout. **Conclusions:** The greater improvement in base excess in the rats receiving more rapidly administered LR suggests a benefit to rapid fluids in this controlled hemorrhage model. The increase in body temperature, likely indicating a better resumption of metabolic processes may also support this. (Support: Pfizer, VA Central IA Health Care Sys, IMMC)1.Knöferl *et al.* Shock 9:(suppl):49,1998.

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EFFECTS OF DIFFERENT HYPERTONIC RESUSCITATION FLUIDS ON ANTIOXIDANT STATUS OF BRAINS FROM RATS SUBJECTED TO TRAUMATIC BRAIN INJURY (TBI) AND HEMORRHAGE (H). M.A. Dubick*, D.S. DeWitt*, R.L. Villarreal*, J. Thomas* and G.C. Kramer. US Army Inst. Surg. Res., San Antonio, TX 78234 and Univ Texas Med Br, Galveston, TX 77550.

Traumatic brain injury increases the morbidity and mortality associated with hemorrhagic shock, and both conditions are known to generate reactive oxygen species (ROS). The present study investigated the effects of small volume hypertonic resuscitation on brain antioxidant status in a rat model of TBI-H. Anesthetized rats were subjected to fluid percussion brain injury (2.5 ata) and after 5 min, were hemorrhaged to a MAP of 50 mmHg over 15 min. After 30 min, groups of rats (n=3-6/gp) were resuscitated with 48 ml/kg of lactated Ringer's (LR) or 6 ml/kg of 7.5% hypertonic saline fluids containing either acetate dextran (HAD), arginine (HArg), or Arg-αα-hemoglobin (HArg-Hb). Rats were euthanatized 2 hr later and brains harvested. Brain thiobarbituric acid reactive substances (TBARS) in TBI-H rats resuscitated with LR were over 3-fold higher than uninjured controls (132±11 vs 42±3 nmol/g; $p<0.05$), while manganese superoxide dismutase activity in LR treated rats was about 50% of control levels (102±5 vs 198±5 U/g; $p<0.05$). Neither HAD, HArg, nor HArg-Hb modified these responses to TBI-H. Interestingly, the presence of Hb in the resuscitation fluid did not augment ROS generation in response to TBI-H. These data support the hypothesis that TBI-H induces ROS generation and that these hypertonic formulations do not ameliorate oxidative injury. The addition of antioxidants to the resuscitation fluid warrants investigation.

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USE OF OXYGEN POST HEMORRHAGE AND THE EFFECT OF SELECTIVE V_1 BLOCKADE OF ARGININE VASOPRESSIN. R. Gunther, H. Ho, G. Helbig*, and J. Davis*. Dept. of Surgery, Sch. of Med., Univ. of Calif., Davis, 95616.

Arginine vasopressin (AVP) levels are greatly increased with burn and hemorrhagic shock. Blockade of AVP V_1 receptors in burn shock increased cardiac output (CO). We investigated the relation between CO, heart rate (HR), and mean aortic pressure (MAP) in rats that were treated with oxygen during the post hemorrhage period with and without AVP blockade. Rats were anesthetized with 1.5 to 2.0% isoflurane in air and divided into a control (C), vehicle only, and a treatment (T) group given a selective V_1 blocker as a bolus (10 μg/kg) at time zero and again at 50 min (5μg/kg). A 13.1 ml/kg fixed volume hemorrhage over 70 min was used (**Hem**). CO was by thermal dilution. At 30 min post hemorrhage (**Air**), both groups were switched to 100% oxygen for an additional 30 min (O_2). Data are mean ± SEM (*= $P \leq 0.05$ within and ** = between groups).

		Control	Hem	Air	O_2
MAP mmHg	C	75±2	46±4*	55±4*	64±5
	T	81±9	50±4*	59±5*	77±1**
HR ppm	C	334±12	290±13*	302±10*	304±15*
	T	338±18	313±8	324±6	319±11

Map was significantly greater in T group with O_2. CO was not different between groups while the drop in HR was blocked in T group. We conclude that V1 blockade had a direct effect on the heart and improved MAP with O_2.

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POLYMERIZATION OF ALBUMIN DOES NOT IMPROVE ITS RESUSCITATIVE ACTIONS FOLLOWING HEMORRHAGIC SHOCK, WHEN COMPARED TO NON-POLYMERIZED ALBUMIN. C. Haney* [b], S. Mehendale* [a], P. Buehler [a], and A. Gulati* [a,b]. Departments of Pharmaceutics & Pharmacodynamics [a] and Bioengineering [b], The University of Illinois at Chicago, Chicago, IL 60612

Although the mechanism(s) for plasma volume expansion by albumin is not clear, it is clear that there is a net plasma protein loss following resuscitation with colloids and crystalloids. Objective: We have polymerized commercially available human serum albumin (68 kDa), using Cyclic-Diethylenetriaminepentaacetic acid (DTPA) anhydride, with the intention of improving vascular retention time. Hence, reduced extravasation should improve the duration of plasma volume expansion. Using laser light scattering the polymerized albumin has an average molecular weight of 211 kDa. Methods: Male rats were anesthetized with urethane and bled to a mean arterial pressure (MAP) of 35 mmHg, maintained for 30 min. Animals were randomized into two resuscitation groups (n=4 ea., 100% of the shed blood volume): I) 3 g/dL polymerized albumin and II) 3 g/dL non-polymerized albumin. Results: MAP returned to 96% and 98% of baseline value in the polymerized and non-polymerized groups, respectively at the end of infusion. At 30 min. post infusion, both groups followed a similar decline in MAP. Additionally, there was no significant difference in dP/dt-max at 30 min post infusion, 5226.5 ± 332 and 4788 ± 420 [mmHg/s], group I and II respectively. Resuscitation with polymerized albumin improved the base deficit from hemorrhage by 47% while the non-polymerized group improved only by 36%, which was not significant. All animals were sacrificed at 6 hours.

MAP Profile Over 6 Hours (Mean ± SE)

	Baseline	Hem.	30 min	1 hr	3 hr	6 hr
I	96.8 ± 4.8	35.0 ± 0.6	94.2 ± 1.9	76.7 ± 2.5	49.5 ± 7.7	26.5 ± 9.8
II	91.3 ± 3.5	33.6 ± 1.9	91.7 ± 0.7	70.9 ± 7.6	55.6 ± 4.2	35.5 ± 6.6

Hemodynamics (Mean ± SE)

	CO/kg [mL/min/kg]		TPR/Kg [mmHg/mL/min/kg]		Heart Rate [beat/min]	
	Hem.	60 Min	Hem.	60 Min	Hem.	60 Min
I	70 ± 11	191 ± 26	501 ± 44	426 ± 65	209 ± 40	309 ± 13
II	62 ± 5	232 ± 19	520 ± 63	318 ± 55	261 ± 34	283 ± 24

Conclusion: Polymerization of albumin appears to have no advantage when compared to non-polymerized albumin at the same dose.

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HEMORRHAGIC SHOCK DECREASES HEPATIC CYTOCHROME P450 EXPRESSION.

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Cytochrome P450 enzymes are critical in the metabolism of physiological substrates and the formation of biologically active endogenous compounds. Many drugs are either cleared from the circulation or are converted to active metabolites by the P450 system. A variety of exogenous and endogenous compounds can alter P450 activity including proinflammatory cytokines, steroids, and hormones produced during systemic stress. We have shown that in severely injured trauma patients, P450 enzyme activity is markedly reduced but the mechanisms responsible for this reduced activity are unknown. Hemorrhagic shock is frequently seen in severely injured patients but its effect on P450 expression has not been established. To determine if hemorrhagic shock alters P450 expression, rats were hemorrhaged to a MAP=40 mm Hg for 2.5 hours and then resuscitated with shed blood plus two times the shed blood volume in crystalloid. Animals were sacrificed 4 and 24 hours after resuscitation and hepatic P450 mRNA expression measured by Northern blot analysis. Twenty-four hours after resuscitation from hemorrhagic shock, P450 mRNA levels were suppressed compared to normal control rats and rats sacrificed after 4 hours (see Table). These data demonstrate that hemorrhagic shock profoundly suppresses the mRNA expression of hepatic P450 and that the degree of suppression varies between P450 isoforms. These data also suggest that hemorrhagic shock may contribute to the decreased P450 activity seen in trauma patients.

P450	Normal	Shock-4H	Shock 24H
2E1	100.0±3.2	43.5±45.2	3.8±3.8
1A1	100.0±2.4	4.0±17.1	26.6±9.2 [1,2]
1A2	100.0±8.7	14.4±22.4	29.6±14.2 [2]
2C11	100.0±2.8	4.3±16.5	3.2±1.4 [1,2]

1 $p<0.05$ vs normal 2 $p<0.05$ vs shock-4H

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EFFECTS OF MILD HYPOTHERMIA ON SERUM CYTOKINES AND OUTCOME IN UNCONTROLLED HEMORRHAGIC SHOCK (UHS) IN RATS. R. Kentner*, L. Khan*, S. Tisherman*, F. Rollwagen*, P. Safar. Safar Center for Resuscitation Research, University of Pittsburgh, PA and USUHS, Bethesda, MD, USA.

Pro-inflammatory cytokines are mediators of multiple organ failure after trauma and hemorrhage. We hypothesized that mild hypothermia (34°C), which improved outcome in our UHS rat model, affects beneficially the pro- and counter-inflammatory cytokine responses. **Methods:** UHS phase I was induced by blood withdrawal of 3ml/100g followed by tail amputation. Hypotensive fluid resuscitation (FR) to MAP 40 mm Hg with blood was started at 30 min and continued to UHS 90 min. Resuscitation phase II was with hemostasis and FR with blood and LR. Observation phase III was to 72h. Groups were normothermia and mild hypothermia (from 30 min until 150 min) (n=10 each), plus 3 shams. Blood samples were taken at baseline (BL), 150 min, and days 1, 2, 3. Serum IL-1β, IL-6, IL-10 and TNF-α were analyzed with rat ELISA test kits. Values are expressed as median and 25/75 percentiles. **Results:** Survival to 72h was better in the hypothermia group (6/10) vs. the normothermia group (1/10) ($p=0.04$). All cytokine levels increased from BL to 150 min in both groups ($p<0.01$). In the normothermia vs. hypothermia groups, at 150 min, IL-1β levels were 185(89-411) vs. 96(51-141) pg/ml ($p=0.15$). IL-6 levels were 2242(1842-4642) vs. 1745(541-2946) pg/ml ($p=0.20$). In contrast, TNF-α levels were 97 pg/ml(72-212) vs. 394(232-476)($p=0.02$), and IL-10 levels were 1.7 pg/ml(0-13.5) vs. 15.8(1.4-24.8)($p=0.09$) in the normothermia vs. hypothermia groups. IL-10 remained elevated until 72h. High IL-1β levels (>100 pg/ml) at 150 min were associated with poor outcome (odds ratio 66, C.I. 3.5-1255). In shams there was no increase of cytokines compared to baseline. **Conclusion**: Mild hypothermia during UHS, which improves outcome, seems to decrease pro-inflammatory and increase counter-inflammatory cytokine release. Monitoring cytokine levels might help in titrating hypothermia.

(Supported by the Office of Naval Research, USA)

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FEMALE SEX HORMONES REGULATE TNF-α PRODUCTION AFTER TRAUMA-HEMORRHAGE.
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Previous studies have shown that female sex hormones protect against immune depression and increased susceptibility to sepsis after trauma-hemorrhage. However, the role of pro-inflammatory cytokines and Kupffer cells (KC) in this process in unknown. To study this, ovariectomy (OVX), to decrease systemic female sex hormone levels, or sham-OVX was performed in 8 wk old female C3H/HeN mice. Two wk thereafter, OVX and proestrus sham-OVX (PRO) mice were subjected to laparotomy (i.e., soft tissue trauma) and hemorrhagic shock (35±5 mmHg for 90 min and resuscitation) or sham operation. Plasma and KC were harvested at 2 hr post-Hem and plasma TNF-α and IL-6 levels along with KC production of these cytokines was

	Plasma		*Kupffer cells*	
	TNF-α (U/ml)	IL-6 (U/ml)	TNF-α (U/ml)	IL-6 (U/ml)
Sham				
PRO	13.2±2.4	0±0	0.1±0.1	0±0
OVX	3.1±1.3	0±0	0.1±0.1	0±0
Hem				
PRO	16.9±3.3	18±8*	0.2±0.1	3±1*
OVX	23.2±4.9†	18±7†	0.7±0.2†	3±1†

*(n=7-8/group), mean±SEM, One way ANOVA, *p<0.05 vs. Sham PRO, †p<0.05 vs. Sham OVX*

determined. Plasma TNF-α levels and KC TNF-α production after Hem were only increased in OVX females. In contrast, plasma IL-6 levels and KC IL-6 production were increased in both groups following Hem. The lack of an effect of OVX on IL-6 levels post-Hem may be due to the early time point measured post-Hem (2 hr). Nonetheless, female sex hormones suppressed the elaboration of TNF-α following trauma-hemorrhage which may in part explain the lack of immune depression and increased susceptibility to subsequent sepsis in proestrus females under such conditions. (NIH GM37127).

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CRYSTALLOID AND COLLOID RESUSCITATION OF UNCONTROLLED HEMORRHAGIC SHOCK FOLLOWING MASSIVE SPLENIC INJURY.
Michael M. Krausz, Yulia Bashenko, Mark Hirsh. Dept of Surgery A, Rambam Medical Center, Technion, Haifa, Israel.

Using a standardized massive splenic injury (MSI) model of uncontrolled hemorrhagic shock, we studied the effect of vigorous crystalloid or colloid fluid resuscitation on the hemodynamic response, and survival in rats. The animals were randomized into six groups: gr.1 (n = 8) sham-operated, gr.2 (n = 11) MSI untreated, gr. 3 (n = 10) MSI treated with 41.5 mL/kg Ringer's lactate (LVRL), gr. 4 (n = 13) MSI treated with 5 mL/kg 7.5% NaCl (HTS), gr. 5 (n = 10) MSI treated with 7.5 mL/kg hydroxyethyl starch (HES-7.5), gr.6 (n = 11) MSI treated with 15 mL/kg hydroxyethyl starch (HES-15).

Results: Following MSI mean arterial pressure (MAP) in gr. 2 decreased to 49.5 ± 10.5 mmHg ($p< 0.001$) after 60 min. Mean survival time (MST) was 122.3 ± 17.4 min., and total blood loss (TBL) was 32.9 ± 3.3% of blood volume. LVRL infusion resulted in a MST of 82.5 ± 18.2 min. ($p< 0.01$), and TBL of 53.7 ±2.9% ($p<0.01$). TBL following HTS infusion was 34.1 ± 3.9% and MST was 119.2 ± 19.1 min. HES-7.5 infusion increased TBL to 44.2 ±3.9% ($p<0.05$), but MST remained unchanged. HES-15 infusion resulted in an increase in TBL to 47.8 ±7.1% (0.01), and MST decreased to 100.7 ± 12.3 min. ($p<0.05$).

Conclusion: vigorous infusion of LVRL or HES-15 following MSI resulted in a significant increase in intra-abdominal bleeding and shortened survival time compared to untreated, small volume HTS, or HES-7.5 treated animals.

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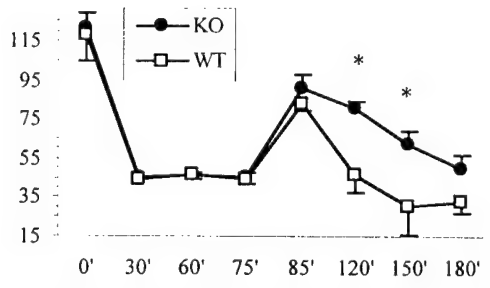
GENETIC DISRUPTION OF POLY(ADP-RIBOSE) SYNTHETASE IMPROVES OUTCOME IN MURINE HEMORRHAGIC SHOCK
L. Liaudet[1], F. G. Soriano[2], J. G. Mabley[3], A.L. Salzman[1,3] and C. Szabo[2,3]. [1]Division of Pulmonary Biology, Children's Hospital Research Foundation, Cincinnati, OH 45229. [2]Dpt of Surgery, New Jersey Medical School, Newark, NJ 01703. [3]Inotek Corporation, Beverly, MA 01915.

Hemorrhagic shock (HS) and resuscitation (RES) lead to the widespread formation of nitrogen- and oxygen-centered free radicals. A major consequence is the development of DNA damage and activation of the enzyme poly(ADP-ribose) synthetase (PARS). In turn, PARS decreases cellular NAD and ATP, a mechanism which might contribute to the cardiovascular failure and organ dysfunction in HS-RES. We tested this hypothesis in a murine model of HS, using PARS knockout (KO) and wild type (WT) mice. Catheters were placed in a carotid artery (blood pressure) and a femoral artery (blood withdrawal and RES). Mice were bled in 30 min to a mean BP of 45 mm Hg, maintained for 45 min and then resuscitated with isotonic saline (2x vol of shed blood in 10 min). Results:



The decline in BP following RES was blunted in PARS KO animals compared to WT (* $p < 0.05$, *t* test). Also, survival times were increased in PARS KO (277±16 min vs 147±30 min, $p<0.05$). These data support a mechanistic role of PARS in the pathophysiology of HS-RES.

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THE EFFECT OF DIFFERENT VOLUMES OF FLUID RESUSCITATION AFTER COMBINED TRAUMA HEMORRHAGIC SHOCK AT HIGH ALTITUDE

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The purpose of this experiment is to study the effect of different volumes of fluid resuscitation after traumatic hemorrhagic shock at high altitude. Seventy-two Wistar rats, transported to LaSa, Tibet from sea level, were anesthetized with sodium pentobarbital(40mg/kg,ip), traumatic hemorrhagic shock was induced by right-femur fracture followed by bleeding to 45 mmHg of mean arterial pressure(MAP) for 1 hour. Then, they were infused one volume, 1.5 , 2 and 3 volumes lactated Ringer's(LR) solution of shed blood respectively. Rats bled and injured but not received infusion served as control group. The observed parameters include MAP, left intraventricular systolic pressure(LVSP) , ± dp/dtmax , water content of lung and brain, and the survival time. The results indicated that the 1 and 1.5 volume LR solution infusion effectively resuscitated traumatic hemorrhagic shock at high altitude. MAP increased 43% and 27% , LVSP increased 56% and 44%, +dp/dtmax increased 53% and 59% respectively. The survival time was prolonged to 17.8h±6.8h and 14.9h±11.2h from 8.2h±10.2h in control group ,$P<0.05$. The water content of lung and brain did not increase. Two and 3 volumes LR solution did not effectively resuscitate shocked rats. MAP, LVSP and ± dp/dtmax did not increase. Water content of lung and brain increased compared to control group.Survival time was not prolonged (7.2h±3.48h and 7.2h±3.29h respectively) compared to 8.2h±10.2h in controls. It was suggested that 1 and 1.5 volume LR solution infusion to resuscitate traumatic hemorrhagic shock at high altitude is more effective and has less side effects than two or more volumes of LR solution infusion.

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EXPLORING SAFETY CONCERNS OF ENALAPRILAT DURING RESUSCITATION.

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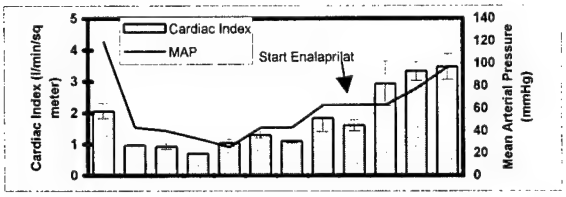
Enalaprilat may be useful for improving GI P_iCO_2 in trauma patients; however, concern over its potential for causing hypotension exists. **Methods:** Five dogs were anesthetized, instrumented, and bled (MAP= 40-45mmHg, then 30-35 mmHg for periods of 30 min). Stepped resuscitation occurred with lactated Ringer's IV at 60ml/kg/hr as a max rate. The resuscitation target MAP's were 40-45mmHg for 30min, 60-65 mmHg for 30min, 60-65mmHg for 30min with a constant rate infusion (CRI) of enalaprilat (0.02 mg/kg/min), then 70-75mmHg with continued enalaprilat. Cardiac index (CI) was measured at the start and end of each interval.

Results: 4 survived hemorrhage. The target MAP was achieved and successfully held in each. Average CI at start of enalaprilat =1.6±0.18, after 30min of enalaprilat with MAP=60-65mmHg CI=2.9±0.71, $p<0.05$.



Conclusions: Starting an enalaprilat CRI during moderate hypotension (MAP 60-65mmHg) does not preclude maintenance or an increase in MAP with a limited fluid rate. Additionally, an enalaprilat CRI can increase CI during moderate hypotension. (Support: Pfizer, VA Central IA Health Care Sys, DM Research & Ed Corp, IA Space Grant Consortium, Eagles)

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PRE-HEMORRHAGE GATORADE® EFFECTS ON GI P_iCO_2.

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We hypothesized that provision of a glucose/electrolyte solution (Gatorade®) rather than fasting pre-bleed might be beneficial for maintaining GI mucosal energy status (theoretically inversely related to GI P_iCO_2) during hemorrhage (H). **Methods:** After 18hr with only water (W), Gatorade® (G), or chow and water (C), Wistar-Furth rats were anesthetized; instrumented (gastric & colonic fiber optic probes Neotrend®); hemorrhaged (MAP= 35-40mmHg >60min until MAP <30mmHg for >10min or <25mmHg for >1min, or 120 min elapsed); resuscitated (R) for 3hr with lactated Ringer's (60ml/hr as needed, MAP=75-80mmHg); then euthanized. **Results:** Mean±SEM are reported (mmHg).

	start H	start R	1hr R	2hr R	3hr R
W gP_iCO_2	89±18	78±15	86±9	65±7	54±14
W cP_iCO_2	68±5	76±3	58±6	51±2	50±2
G gP_iCO_2	102±18	156±25	132±21	110±17	93±19
G cP_iCO_2	64±2	74±8	59±3	57±3	50±3
C gP_iCO_2	106±12	113±13	82±6	82±12	81±11
C cP_iCO_2	77±7	106±7	68±4	64±4	66±10

Abbreviations: g=gastric, c=colonic, i=intraluminal. 6 of 12 W, 7 of 12 G, and 13 of 15 C rats survived to euthanasia. **Conclusions:** Gastric P_iCO_2 was highest and underwent the greatest change in the Gatorade® rats ($p<0.05$). Colonic P_iCO_2 was similar in the Gatorade® and fasted rats and higher in the chow rats ($p<0.05$). According to the P_iCO_2 data, fasting protected gastric and colonic mucosal energy status, and Gatorade® exacerbated the effect of hemorrhage on gastric mucosal energy status but not colonic mucosal energy status. Why this occurred requires further investigation. (Support: Pfizer, VA Central IA Health Care System, Drake U, Diametrics)

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TRAUMA SHOCK RESUSCITATION: WHAT WORKS

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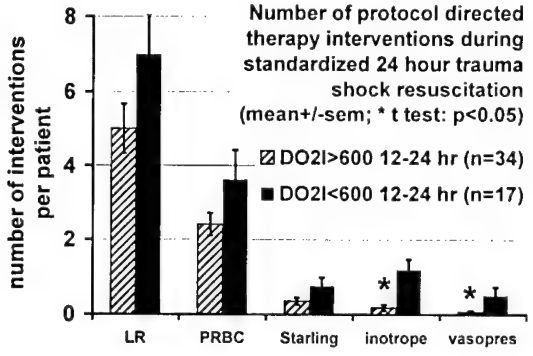
We implemented a standardized protocol for trauma shock resuscitation with prospective data collection. The protocol comprises a hierarchy of 5 therapies (with intervention thresholds) to achieve $DO_2I \geq 600$ mL/min/m^2 goal for 24 hrs: LR (lactated Ringer's; pulmonary capillary wedge pressure (PCWP)<15 mmHg), blood (PRBC; Hb<10 g/dL), Starling curve to optimize cardiac index (CI)-PCWP (Hb>10, PCWP>15 and DO2I<600), inotrope (CI-PCWP optimized and DO_2I<600), and vasopressor (MAP<65 mmHg). From Jan-Aug 1999 51 trauma patients were resuscitated using the standardized protocol. We compare the frequency of use of individual therapies in 34 (67%) successful ($DO_2I \geq 600$ during 12-24 hr; ISS 33±3) vs 17 (33%) unsuccessful (DO_2I<600 during 12-24 hr; ISS 34±5) resuscitations.



Successfully resuscitated patients required fewer interventions with inotrope/vasopressor than patients unsuccessful in achieving the standardized protocol performance goal. Inotrope/vasopressor intervention did not achieve $DO_2I \geq 600$. Fluid loading and blood replacement were mainstay therapies that worked.

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FIBER OPTIC P_iCO_2 AT FOUR GASTROINTESTINAL SITES DURING HEMORRHAGE AND RESUSCITATION.

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As clinical gastrointestinal P_iCO_2 monitoring increases, the equivalence of different GI sites for monitoring patient status becomes important. **Methods:** In two hemorrhage (H) and resuscitation (R) studies (different R protocols), fiber optic (Neotrend®) PCO_2 monitoring systems were used simultaneously to measure P_iCO_2 in the esophagus, stomach, and duodenum (5 dogs, 1st study) and in the stomach, duodenum, and ileum (10 dogs, 2nd study). **Results:** During H, P_iCO_2 increased at all sites in both studies, but the magnitude of the increases varied. No site was consistently the highest. During R, significant differences between each of the sites (including direction of change) existed in 3 of the 4 surviving dogs (1st study, $p \leq 0.037$) and 7 of the 8 surviving dogs (2nd study, $p<0.05$). The other 2 dogs approached significance ($p=0.057$ 1st study and $p=0.081$ 2nd study). During R, duodenal P_iCO_2 levels were topped out in 5 of the 8 surviving dogs (2nd study). **Conclusions:** Various sites GI P_iCO_2 monitoring sites may be acceptable for aiding initial severity of shock assessment. During resuscitation, however, different GI sites do not even necessarily trend in the same direction. Thus, the energy status in a specific GI site during resuscitation cannot be assumed to indicate the energy status of any other GI site. Which site has the tightest link between its energy status and the eventual outcome of the patient awaits determination. (Support: Pfizer, VA Central IA Health Care Sys, DM Research & Ed Corp, IA Space Grant Consortium, Diametrics)

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BLOOD LOSS FOLLOWING FLUID RESUSCITATION IN UNCONTROLLED HEMORRHAGE MODELS SIMULATING PENETRATING AND BLUNT ABDOMINAL TRAUMA.

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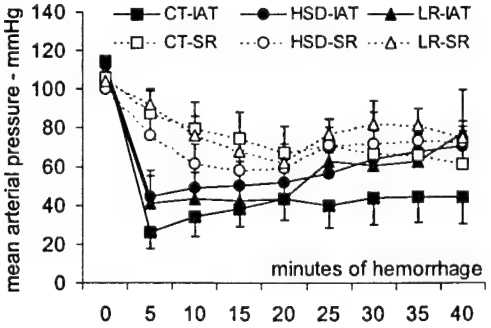
It has been suggested that prehospital fluid resuscitation for hypotensive patients sustaining abdominal trauma may increase blood loss. Methods: Anesthetized dogs (17±2 kg), were submitted to two distinct models of uncontrolled intraabdominal hemorrhage. Suture lines were placed either around the spleen or through the left common iliac artery, and exteriorized. After abdominal closure, splenic rupture with hilar vascular injury (SR, n=30) or a 3-mm iliac arterial tear (IAT, n=18) was produced by pulling the exteriorized line, and animals were randomized into three groups 20 min later: lactated Ringers (LR), 32 ml/kg over 15 min; 7,5% NaCl/6% Dextran70 (HSD), 4 ml/kg over 4 min, or controls (CT), no fluids. Results: Both HSD and LR treatments restored cardiac output, while in controls it remained reduced. No significant differences occurred in blood loss (mL/kg) between CT and treated animals after IAT (CT=48±6; HSD=42±2; LR=49±1), or SR (CT=38±4; HSD=43±5; LR=42±5). Conclusion: No fluid infusion during intraabdominal bleeding resulted in a low blood flow state, while resuscitation with both HSD and LR produced hemodynamic benefits without increased blood loss.



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EFFECT OF ENALAPRILAT ON GASTROINTESTINAL INTRALUMINAL PCO_2 DURING HYPOTENSIVE RESUSCITATION. T. Rickers*, P. Wall, L. Henderson*, C. Buising*, A. Cárdenas*, F. Raymond*, D. Hoganson*, C. Brunkan*, J. Gale*, JM. Langley*, L. Owens*, D. Moorman*, G. Timberlake. Surg Ed & Trauma, IA Methodist Med Ctr, and Drake Univ., Des Moines, IA 50311.

Interventions which improve GI mucosal energy status (indicated by a decreasing GI P_iCO_2) may help improve outcomes in trauma patients. Enalaprilat may improve splanchnic perfusion, thus improving mucosal energy status. **Methods:** Ten dogs were anesthetized, instrumented (including Neotrend® fiber optic probes for gastric, duodenal, and ileal P_iCO_2), and bled (MAP=40-45mmHg for 30min then 30-35mmHg for 30min). Lactated Ringer's was administered as needed (rate ≤ 60ml/kg/hr) throughout hypotensive resuscitation (MAP=40-45mmHg for 150min). A constant rate infusion (CRI) of enalaprilat (0.02 mg/kg/hr, n=5) or saline (n=5) was started 30min into resuscitation. **Results:** 4 dogs survived hemorrhage in each group. Results are averages ±SEM.

	Start CRI PiCO2 (mmHg)			*End Resus. PiCO2 (mmHg)*		
	Gastric	Duod	Ileal	Gastric	Duod	Ileal
Enal.	178±17	199±0	189±10	153±33	199±0	185±21
Saline	113±31	177±22	109±31	126±31	146±52	97±31

The change in P_iCO_2 was gastric: 12±17 vs -17±21, duodenal: -35±23 vs 0±0, ileal: -12±3 vs 8.5±6 in saline and enalaprilat dogs, respectively. **Conclusions:** No significant decrease in P_iCO_2 at any site occurred with enalaprilat as compared to saline. A drawback of this study was the topping out of the Neotrend® probe at 199mmHg. This occurred in the duodenum of all enalaprilat dogs (n=4) vs one saline (n=1); the stomach (n=2) vs (n=1); and the ileum (n=2) vs (n=0). It is also possible that, given more time, differences may have developed between the groups. (Support: Pfizer, VA Central IA Health Care Sys, DM Research & Ed Corp, IA Space Grant Consortium, Diametrics, Arrow Int'l)

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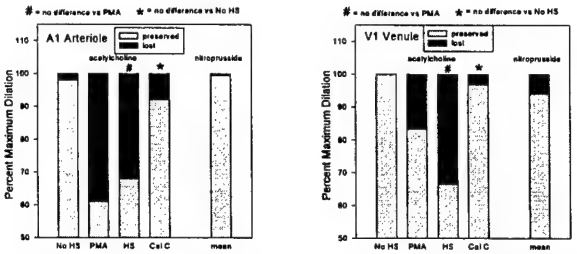
PROTEIN KINASE C INHIBITION PREVENTS MESENTERIC ENDOTHELIAL DYSFUNCTION AFTER RESUSCITATED HEMORRHAGIC SHOCK. B Ryu*,T Ferrario*,W. Calvo* and W. Flynn,Jr Dept. of Surgery, SUNY Buffalo, Buffalo, NY 14215

Ischemia reperfusion increases protein kinase C (PKC) activity. This study was performed to determine the contribution of PKC activity to mesenteric endothelial dysfunction after resuscitated hemorrhagic shock. Rat ilem was prepared for intravital microscopy with its neurovasculature intact. Groups included no hemorrhage (No HS, saline), phorbol ester (PMA, 5 x 10^{-7} mol/l ,PKC agonist), HS (hemorrhage to 50% of baseline blood pressure for 60 minutes and resuscitation) and Cal C (calphostin C; PKC inhibitor, 5 x 10^{-7} mol/l during recuscitation). The endothelial dependent dilator acetylcholine (Ach 10^{-5} mol/l) and independent dilator nitroprusside (SNP 10^{-5} mol/l) were applied topically. First order arteries (A1) and veins (V1) are reported as % maximum dilation 60 minutes after resuscitation



Hemorrhage and resuscitation cause endothelial dysfunction in arterial and venous microcirculations. Blockade of PKC activity with cal C prevents this dysfunction suggesting that PKC activity contributes to the endothelial dysfunction seen in the mesenteric microcirculation after resuscitated hemorrhagic shock.

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TITRATED HYPERTONIC/HYPERONCOTIC SOLUTION (HHS) REDUCES INFUSION VOLUME REQUIREMENT DURING UNCONTROLLED HEMORRHAGIC SHOCK (UHS) IN RATS. P. Safar, R. Kentner*, S. Prueckner*, W. Behringer*, X. Wu*, S. A. Tisherman*. Safar Center for Resuscitation Research, University of Pittsburgh, PA, USA.

A small *bolus* of HHS infused for resuscitation from *controlled* HS has been found to be effective. We hypothesized that a *titrated* i.v. infusion of HHS for hypotensive fluid resuscitation (FR) (to MAP=40 mm Hg) during *uncontrolled* HS compared to lactated Ringer's solution (LR) increases survival, reduces fluid requirement and decreases liver dysfunction without increasing blood loss. **Methods:** We used our 3-phased UHS outcome model in rats. Blood withdrawal of 3ml/100g over 15 min was followed by tail amputation. Hypotensive FR with HHS or LR was started at UHS 20 min and continued to UHS 90 min. Then, hemostasis and allout FR was with shed blood and LR until 270 min. Outcome was observed to 72 h. Liver dysoxia was monitored as increase in liver surface pCO_2. Serum electrolytes and blood gases were analyzed. Values are expressed as median and 25/75 percentiles. **Results**: During UHS, fluid requirements were 5.4 ml/kg (2.8-6.4) in the HHS group vs. 50.1 ml/kg (12.2-106.6) in the LR group ($p=0.0003$). HHS did not increase blood loss, 12.9 ml/kg (9.7-19.8) with HHS vs. 13.0(5.6-20.1) with LR. Serum sodium concentrations, moderately elevated in the HHS group, were 152 mmol/l (147-153) vs. 140 (139-143) ($p<0.01$). Liver dysoxia increased during UHS ($p<0.01$) without difference between groups. HHS vs. LR did not improve 72h survival rates (3/10 vs. 2/10). Median survival times were 690 min(210-∞) with HHS vs. 330 min(150-2670) with LR ($p=0.75$). **Conclusion**: In rats with UHS, a titrated infusion of HHS can maintain controlled hypotension (prevent cardiac arrest) with only 1/10th of the volume needed of LR, without increasing blood loss or changing survival times and rates. (Supported by the Office of Naval Research, USA)

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HEMORRHAGE-INDUCED LATE MYOCARDIAL TNF-α EXPRESSION RESULTS FROM *DE NOVO* PROTEIN SYNTHESIS. R. Shahani, S. Nicholson, L. Klein, B. Rubin, P. Walker, T. Lindsay. Toronto General Hospital and University of Toronto, Toronto, ON M5G 2C4.

Hemorrhagic shock (HS) is known to induce myocardial contractile dysfunction. Hemorrhage induces the translocation

of the transcription factor, NF-κB to the nucleus and stimulates significant myocardial TNF-α expression. Our investigations have shown that TNF-α expression rises significantly within 30 minutes of HS and reaches a maximum following 1 hour of HS and 45 minutes of resuscitation. TNF-α mediates 80% of the hemorrhage-induced cardiac dysfunction. Both pre-formed and newly synthesized TNF-α may account for the myocardial TNF-α expression noted following HS. We hypothesized that the rise in myocardial TNF-α levels results from *de novo* protein synthesis. HS was induced by the withdrawal of blood to a mean BP of 50 mmHg. Animals were fully resuscitated with shed blood and lactated Ringer's to return mean BP to pre-shock levels. ELISA was used to quantify myocardial TNF-α expression. The protein synthesis inhibitor, cycloheximide (1 mg/kg), was administered 1 hour prior to hemorrhage.

Time	Sham	HS	HS + Cyclo
30 min HS	34.45 ± 1.94	231.18 ± 19.24*	162.13 ± 7.49#
1 hr HS/45 min resuscitation	39.64 ± 4.25	393.16 ± 21.62*	54.16 ± 6.12

Table 1. Myocardial TNF-α levels (pg/mg protein) following hemorrhagic shock (HS) with cycloheximide (Cyclo) at 30 minutes of HS and 1 hour of HS and 45 minutes of resuscitation. All values are Mean ± SEM. *p<0.05 vs. Sham and HS + Cyclo. #p<0.05 vs. Sham.

After 30 minutes of HS, myocardial TNF-α levels rose 6-fold while cycloheximide pre-treatment reduced myocardial TNF-α by 30%. However, following 1 hour of HS and 45 minutes of resuscitation where myocardial reached a maximum of 393.16 pg/mg protein, protein synthesis inhibition reduced myocardial TNF-α levels by 87%. Thus, the early rise in myocardial TNF-α levels is less dependent upon protein synthesis than the peak increase noted in the heart following 1 hour of HS and 45 minutes of resuscitation. Therefore, preformed stores of TNF-α may be the source for the early rise in myocardial TNF-α noted during HS.

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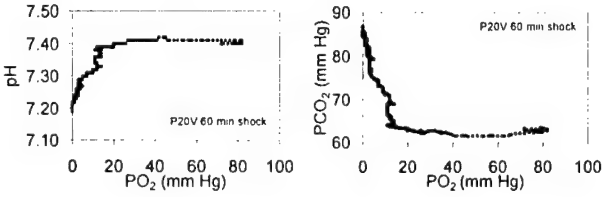
REGIONAL HEPATIC DYSOXIA DURING HEMORRHAGIC SHOCK IN SWINE. B. Soller*, S. Heard, N. Cingo*, J. Puyana. UMass Medical School, Worcester, MA 01655 and Brigham & Women's Hospital, Boston, MA 02115

The study of hepatic dysoxia (O_2 supply insufficeint to meet demand) is complicated because of the liver's dual blood supply and the interrelationship of flow in the hepatic artery and portal vein. We investigated the use of a fiberoptic sensor to measure regional tissue levels of PO_2, PCO_2 and pH in the liver. **Methods:** After performing a laparotomy, a 0.5 mm fiberoptic sensor was placed ≈5 mm deep in one hepatic lobe. Ten animals were randomly assigned to either 30, 60 or 90 minutes of shock at systolic blood pressure of 40 mm Hg. Three animals were instrumented and monitored for 90 min without shock. Tissue PO_2, PCO_2 and pH were recorded every 10 sec. PCO_2 and pH were plotted against PO_2 during the shock period and the plots were examined for a biphasic relationship; if present, critical levels of PCO_2, pH and PO_2 were identified. **Results:** None of the sham animals showed a biphasic relationship between PO_2 and either pH or PCO_2. All 10 shock animals showed a biphasic response, including 2 animals that died during shock. Critical values were determined to be: pH_{crit} = 7.32 ± 0.02, PCO_{2crit} = 61 ± 4, PO_{2crit} = 25 ± 10. Four animals had PO_{2crit} = 0. **Conclusions:** Regional dysoxia was present in the swine liver during hemorrhagic shock and can be simply detected with a fiberoptic sensor.



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EFFECTS OF LIMITED RESUSCITATION WITH 7.5% NaCl/6% DEXTRAN-70 (HSD) IN A TRAUMATIC BRAIN INJURY /UNCONTROLLED HEMORRHAGE MODEL S.Stern B. Zink* M. Mertz* X Wang* Univ of Michigan, Ann Arbor , MI, 48109-0303.

Limited resuscitation (LRES) of combined traumatic brain injury (TBI) and uncontrolled hemorrhage (UH) reduces hemorrhage volume and short-term mortality, but at the expense of cerebral perfusion. Studies of resuscitation with HSD demonstrate enhanced cerebral blood flow (CBF) and O_2 delivery (O_2 del) as compared to resuscitation with 0.9%NaCl (NS). Hypothesis: In a model of combined fluid percussion TBI (FP-TBI) and UH, LRES with HSD will provide the most optimal cerebrovascular profile while minimizing hemorrhage volume as compared to either standard resuscitation (SR) or LRES with NS. Methods: 35 swine (18-24 kg) underwent 3.0 atm FP-TBI and were hemorrhaged to a MAP=30mmHg in the presence of a 3mm aortic tear. Groups I (N=14) and II (N=9) were resuscitated with NS (6mL/kg/min); Group III (N=12) with HSD (.72mL/kg/min). Fluids were initially infused as needed to maintain a goal MAP=60mmHg (Grps I&III) or 80mmHg (Grp II). After 60 min, the aorta was clamped and animals were resuscitated to normal physiologic levels and observed for 150 min. CBF was measured using colored microspheres. Results: Mortality was 31%, 56%, and 0% (Fisher exact; P=0.015), while hemorrhage vol's were 45±19, 74±35, and 38±6 mL/kg (ANOVA; P=0.001) for Grps I, II, and III. Despite initial LRES, Grp III CBF's were not < baseline (Paired t-test; P>0.05), and CPP's remained ≥70mmHg.

	^MinCBF (mL/g/min)	^MinCPP (mmHg)	^MinO2Del (mL/kg/min)
Grp I	.548±.203	52±8	10.03±4.33
Grp III	.808±.325	73±11	14.02±3.08
t-test	*P=0.031	*P<0.001	*P=0.014

^Values are mean minimum measurements during resusc. Conclusion: In the setting of combined TBI and UH, LRES with HSD optimizes hemodynamic and cerebrovascular physiology without accentuating hemorrhage.

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ENTERAL OR ORAL IL-6 DOES NOT IMPROVE SURVIVAL IN RATS DURING HEMORRHAGIC SHOCK S. Tisherman*, X. Wu*, S. Prueckner*, FM. Rollwagen*, J. Stezoski*, P. Safar
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Oral IL-6 alleviated bacteremia and gut epithelial apoptosis after hemorrhagic shock (HS) in mice without

deleterious systemic side-effects. The goal of this study was to explore potential HS survival benefit of oral or enteral IL-6 in rats. In *Study A*, 20 rats (10 controls and 10 IL-6) were anesthetized with spontaneous breathing of halothane and N_2O. HS was initiated with 3 ml/100g blood withdrawal over 15 min, and then MAP maintained at 40 to 50 mmHg. After HS 90 min, resuscitation included reinfusion of shed blood and additional Ringer's solution to restore normotension for 1 h. Rats in the IL-6 group then received 300 units IL-6 via a feeding cannula, while rats in the control group received the same volume of vehicle. There were 7/10 survivors to 72 h in the control group and 5/10 in the IL-6 group (NS). Macroscopic gut necrosis (in non-survivors) was not different between the two groups. In *Study B*, 20 rats were fasted over night, and prepared as in Study A. HS was initiated with 2 ml/100g blood withdrawal over 10 min, and MAP maintained at 35-40 mmHg for 80 min. IL-6 rats received 3,000 units IL-6 in 5 ml normal saline injected directed into the ileal lumen 20 min after induction of shock and again at the end of 1 h resuscitation. Control rats received saline. Survival to 72 h was 6/10 in both groups. In spite of the similar survival rates, *Study B* with fasted rats had a significantly lower frequency of small intestine injury than *Study A* with fed rats (7/20 rats vs. 16/20, $p<0.01$). Conclusion: IL-6, given orally or injected directly into the small intestinal lumen, may not significantly influence survival time and rate following HS in rats. Fasting may significantly alter the gut response to HS.
(Supported by the Office of Naval Research, USA).

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MONITORING INTRALUMINAL PCO2: A COMPARISON OF AIR-FLOW AND FIBER OPTIC METHODS. P. Wall, L. Henderson*, C. Buising*, T. Rickers*, A. Cárdenas*, T. Mattson*, A. Larkin*, L. Wittkopf*, D. Davis*, F. Raymond*, L. Owens*, D. Moorman*, G. Timberlake. Surg Ed & Trauma, IA Methodist Med Ctr, & Drake U, Des Moines, IA 50311.

The clinical use of gastrointestinal intraluminal PCO_2 (P_iCO_2) information is increasing. Therefore, determining the limitations and potential caveats of different gastrointestinal P_iCO_2 monitoring systems is clinically important. **Methods:** Air-flow (Tonocap®) and fiber optic (Neotrend®) PCO_2 monitoring systems were used simultaneously to measure the PCO_2 of humidified air containing 5 and 10% CO_2. The same measuring systems were also used simultaneously to monitor the gastric P_iCO_2 of 15 dogs during hemorrhage (mean arterial pressure 40-45 mmHg for 30 min, then mean arterial pressure 30-35 mmHg for 30 min) and three differing resuscitation protocols. **Results:** Both systems were in agreement *in vitro*. The fiber optic monitoring system, however, provided significantly higher and more rapidly changing gastric P_iCO_2 values with hemorrhage than did the air-flow system. The gastric P_iCO_2 values with the fiber optic and air-flow systems were 69.7 ± 5.0 and 58.6 ± 4.5 mmHg at baseline and 145.8 ± 11.7 and 84.5 ± 6.4 mmHg (mean±SEM, $p<0.05$) at the end of hemorrhage, respectively. **Conclusions:** Despite *in vitro* agreement with fiber optic methods, the use of saline or air-flow based methods for determining gastrointestinal P_iCO_2 may influence the values obtained. Techniques that do not remove samples, such as fiber optic methods, for monitoring gastrointestinal P_iCO_2 are preferable because they neither deliver O_2 to nor remove CO_2 from the local microenvironment. (Support: Pfizer, VA Central IA Health Care Sys, DM Research & Ed Corp, IA Space Grant Consortium, Arrow Int'l, Diametrics)

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HEMORRHAGIC SHOCK AND RESUSCITATION ACTIVATES POLY (ADP-RIBOSE) POLYMERASE IN RAT ILEUM. JA Watts, RM Grattan*, BS Whitlow*, LR Thornton*, RW Barbee. Carolinas Medical Center, Charlotte, NC, 28232

Previous studies provide pharmacological evidence that poly (ADP-ribose) polymerase (PARP) is activated following hemorrhagic shock, but do not directly measure activity or define the time of activation. The present studies measure PARP activity in the initial stages of resuscitation after hemorrhage. Rats were instrumented using isoflurane anesthesia. Awake, non-heparinized, rats were hemorrhaged (1 ml/min) and maintained at 40 mm Hg for 1 hr. A 10 min. resuscitation with Ringer's solution (1 ml/min) followed, and tissues were isolated. Sham animals were time-matched. PARP activity was determined by measuring incorporation of ^{32}-P NAD^+ into protein in nuclear extracts. Blanks contained no extract. Protein content was determined and radioactivity was converted to pmol/min/μg protein. Results: Hemorrhage resulted in severe shock (lactate: 0.7±.07 basal vs 14.4±1.0 shock, pH: 7.43±.02 basal vs 7.09±.07 shock). PARP activity was elevated in the ileum following 10 min of resuscitation compared with sham animals. 3-aminobenzamide (3AB) inhibited the reactions indicating the activity was due to PARP.

Reaction	Shock	Sham
-3AB	59.8±14.7	10.3±1.8*
+3AB	2.6± 2.1**	2.6±1.4**

*$p<0.05$ shock vs sham; **$p<0.05$ −3AB vs +3AB
Activation of PARP occurred very early in a tissue (ileum) that is prone to ischemia and reperfusion after hemorrhage and resuscitation.

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INTRAPERITONEAL (IP) BUT NOT ENTERIC (EN) ADENOSINE IMPROVES SURVIVAL AFTER PROLONGED VOLUME-CONTROLLED HEMORRHAGIC SHOCK (HS) IN RATS. X. Wu*, EK. Jackson*, J. Stezoski*, S. Tisherman*, P. Safar
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Adenosine (AD) possesses several properties which could be valuable in the protection of viscera during and after HS. In order to circumvent its adverse systemic side effects and achieve local effets, we examined the potential benefit of AD administration IP or EN on survival following

HS. Method: 30 rats were anesthetized and instrumented. HS was initiated with 2.75 ml blood/100g blood withdrawal over 15 min. Total HS duration was 120 min. Rats were divided into 3 groups in a blinded and random fashion. Starting at 20 min HS and continuing through 1 h reperfusion, rats in all three groups received both IP lavage and repeated bolus injection into the ileum of normal saline solution. In the IP group, AD 0.1 mM solution was used for IP lavage, while in the EN group, AD 1.0 mM solution was injected into the ileum. After 120 min HS, normotension was restored and maintained for 1 h by reinfusion of shed blood and Ringer's solution. Observation was continued to 72 h. Results: MAP and HR were similar between groups during HS and resuscitation. K^+, lactate, and BUN levels were significantly lower, and arterial pH significantly higher, in the IP and EN groups compared to the control group. Survival to 72 h was significantly greater in the IP group compared to the control group (9/10 vs. 4/10, $p<0.05$). There were 7/10 survivors to 72 h in the EN group (NS). Conclusion: Both IP and EN adenosine produced beneficial physiologic effects, but only IP adenosine significantly improved survival following HS. No systemic circulatory effects were seen. The IP approach to drug administration during HS may allow targeting topically the most vulnerable organ, the gut, with drugs that may have deleterious systemic side effects.
(Supported by the Office of Naval Research, USA)

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HYPERTONIC SALINE AND PENTOXIFYLLINE RESUSCITATION REDUCE LEUKOCYTE ADHERENCE AFTER HEMORRHAGIC SHOCK. M.M. Yada-Langui*, E.A. Anjos-Valotta*, R. Coimbra, P. Sannomiya*, M. Rocha e Silva. Research Division, Heart Institute (InCor), University of São Paulo Medical School and Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Brazil.

Leukocytes play an important role in the inflammatory response following shock and trauma. We compared the effects of the treatment with hypertonic saline (HS), pentoxifylline (PTX) + lactated Ringer's (LR), and LR alone on microcirculation, using intravital microscopy, in hemorrhaged rats. Rats (240-300g) were bled to a mean arterial pressure of 35 mmHg for 1 h and then randomized into 4 groups: LR (3x shed blood; n=12); HS (7,5% NaCl, 4 mL/Kg; n=14); LR + PTX (25 mg/Kg; n=13) and SHAM (no shock, no treatment; n=10). Additionally, total shed blood was reinfused. 2 h after treatment, the internal spermatic fascia was exteriorized and the microcirculation was observed by a closed-circuit TV coupled to a microscope. The number of leukocytes rolling along the venular endothelium, sticking to the vascular wall and migrating cells were determined, and presented in the table.

Groups	Rollers/10min	Adhered cells/100μm venule lenght	Migrated cells (1,000μm²)
Sham	80.70 ± 4.60	0.40 ±0.20	0.90 ± 0.20
HS	83.50 ± 8.20	1.40 ± 0.40	2.30 ± 0.40
LR	73.10 ± 9.90	4.00 ± 0.90 [b]	3.30 ± 0.80 [c]
LR + PTX	126.00 ± 12.40 [a]	1.70 ± 0.30	1.70 ± 0.30

a: $p<0.05$ (LR+PTX vs Sham, HS and LR); **b:** $p<0.05$ (LR vs Sham, HS and PTX) **c:** $p<0.05$ (LR vs Sham)

Conclusions: HS and PTX treated animals significantly reduced the leukocyte adherence after hemorrhagic shock, compared with LR treated animals. LR also presented higher number of migrated cells compared with the sham group. These results support earlier studies which indicated the potential application of HS and PTX in shock therapy.

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NEUTROPHIL ACTIVATION INDUCED BY LACTATED RINGER'S RESUSCITATION CAN NOT BE PREVENTED BY ALTERING THE VOLUME OR RATE OF INFUSION.

Alam HB, Scultetus A, Koustova E, Stanton K, Anderson D, Austin B, and Rhee P. *Uniformed Services University of the Health Sciences, Bethesda, MD 20814.*

Lactated Ringer's (LR) solution has been shown to cause neutrophil activation. Our hypothesis was that the degree of neutrophil activation would depend on the rate and volume of LR infused. **Methods:** 39 female swine (45-60kg) were subjected to a 28ml/Kg hemorrhage over 15 min, kept in shock for 1 hour, and then resuscitated as follows: 1) anesthesia only; 2) hemorrhage only; 3) whole blood -1x blood loss; 4) LR fast rate- 3x blood loss, over 1 hour; 5) LR slow rate- 3x blood loss, over 3 hours; 6) LR low volume- 1x blood loss, over 1 hour; and 7) 7.5% Hypertonic saline (HTS)- 0.3x blood loss, over 1 hour. Whole blood neutrophil assay with flow cytometery was used to determine intracellular oxidative burst activity by staining with dichlorofluracin diacetate. **Results:** Resuscitation with LR caused significant neutrophil activation independently of the volume or the rate of infusion. No significant activation was seen in the animals resuscitated with hypertonic saline or whole blood. **Conclusion**: Neutrophil activation caused by LR resuscitation is independent of the rate or volume infused. However, using either HTS or whole blood for resuscitation can prevent this neutrophil activation.

	Anes-thesia	No Resus.	Blood 1xBL 1hour	LR 3xBL 1 hour	LR 3xBL 3 hours	LR 1xBL 1 hour	HTS .3xBL 1 hour
	n=5	n=5	n=6	n=6	n=6	n=6	n=5
End Shock	101.2± 9.7	95.6± 22.15	**160± 22.9***	162± 23.4	**115± 14.7***	**173± 17.2***	161± 37.5
End Resus	114.3± 10.4	84.8± 17.6	103.7 ±11.8	**284± 63.2***	**158± 15.3***	**248± 31.7***	220± 44.8

Data presented as percent change in neutrophil florescence compared to baseline ±SEM. *$p<0.05$ using t-test compared to baseline. BL= blood loss

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P38 MAPK ACTIVATION INDUCES RAPID CXCR2 SHEDDING IN PMN

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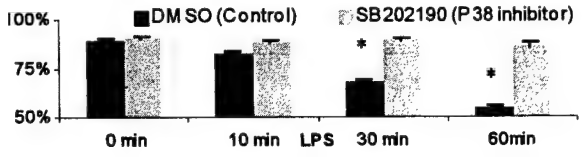
CXC receptors bind chemokines such as IL-8 and play a role in PMN migration, priming, and degranulation. The loss of CXCR2 correlates with an inhibited PMN migration response to various chemotactic stimuli and has been observed following traumatic and septic shock. We have demonstrated that LPS causes activation of the P38 MAPK pathway in human PMN within 5 minutes of stimulation. We hypothesize that LPS-induced P38 MAPK activation induces a rapid shedding of CXCR2. PMN were isolated from healthy volunteers (n=8) as previously described. Cells were pretreated for 1 hour *in vitro*

with the selective P38 inhibitor (SB202190, 1μM) or DMSO control. After pretreatment, PMN were treated with 1μg/mL LPS (E.coli 0111:B4) over a 60 minute time course. CXCR2 was labeled using a receptor-specific antibody and FACS analysis. The percent of cells expressing this receptor and the average receptor density per cell were measured. Statistical significance was determined via ANOVA (*$p<0.02$).



Post-isolation, percent expression of CXCR2 is 94.0±0.6%. LPS induces a rapid decrease in CXCR2 percent expression (**Figure**) and average receptor density per cell within 30 minutes. In PMN pretreated with the P38 pathway inhibitor, SB202190, there was a significant decrease in CXCR2 shedding at 30 and 60 minutes of LPS exposure. In conclusion, these data demonstrate a rapid shedding of PMN CXCR2 in response LPS stimulation *in vitro*. Furthermore, the effect is inhibited by blockade of the P38 MAPK pathway suggesting that activation of the P38 signaling cascade is one mechanism by which PMN shed chemokine receptors.

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TRAUMA / HEMORRHAGE ACTIVATES RAT PMN RESPONSES TO MULTIPLE G-PROTEIN COUPLED CHEMOATTRACTANTS. Z Fekete*, CJ Hauser, JM Adams*, CA Adams*, RM Forsythe*, DZ Xu, JT Sambol*, Q Lu*, D Anjaria*, DH Livingston and EA Deitch.
UMDNJ, Newark, NJ.

BACKGROUND: Studies of cell calcium in rat PMN are rare, and existing studies suggest calcium values markedly different from those seen in human PMN. Moreover, studies of $[Ca^{2+}]_i$ mobilization in circulating rat PMN after hemorrhagic shock are not reported. We developed methods to collect, separate and fura 2-am load circulating rat PMN. We then studied responses to G-protein coupled agonists in PMN isolated from animals subjected to trauma and hemorrhagic shock.

METHODS: Anesthetized rats underwent a 5 cm laparotomy (trauma) and hemorrhagic shock (MAP 30 mmHg x 90 min) with blood collection at 3-6 hours by cardiac puncture (**T/HS**). Control rats (**CON**) underwent only anesthesia and cardiac puncture. PMN were separated using a modified one-stage density gradient separation. Cells were loaded with fura in the presence of sulfinpyrazone and pluronic acid. PMN were stimulated with 1 nM each of recombinant rat GRO, rat MIP-2, or PAF. $[Ca^{2+}]_i$ was measured by spectrofluorometry.

RESULTS: T/HS elevated basal PMN calcium compared to values in **CON** rats (66±5 vs 53±2 nM, $p<0.01$, unpaired *t*-test.) **T/HS** also markedly elevated the peak PMN $[Ca^{2+}]_i$ responses to GRO (165±23 vs. 93±8 nM, $p<0.006$), to MIP-2 (208±24 vs. 95±6 nM, $p<0.0002$) and to PAF (194±26 vs.112±10 nM, $p<0.005$) when compared to **CON** rat responses.

CONCLUSION: After trauma and hemorrhagic shock, rat PMN demonstrate enhanced basal $[Ca^{2+}]_i$. They also manifest markedly enhanced peak $[Ca^{2+}]_i$ responses to a variety of G-protein coupled agonists known to play important roles in the systemic inflammatory response to shock, trauma and sepsis. Using these methods, rat PMN can be shown to demonstrate basal and primed G-protein responses very similar to those seen in human PMN responding to homologous agonists.

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TREATMENT OF BURN-INJURED RATS WITH ANTI-CINC PREVENTS UP-REGULATION OF Ca^{2+} SIGNALING. S. Khan*, N. Fazal*, M. Shamim*, S. Namak*, M. A Choudhry, and M. M. Sayeed.
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CXCs, e.g. CINC (cytokine induced neutrophil chemoattractant) bearing the N-terminal glu-lue-arg (ELR) motif are important neutrophil activator and chemoattractants. Neutrophil activation and subsequent responses to chemoattractants apparently lead to neutrophil hyperresponsiveness in early stages after thermal injury. Elevated plasma CINC in the burn injured rats probably plays a crucial role in PMN signaling up-regulation. We have studied cytosolic $[Ca^{2+}]$ responses to fmlp in neutrophils harvested from sham, burn (25% TBSA skin scald, day 1 post-burn), and anti-CINC antibody treated burn rats. Cytosolic $[Ca^{2+}]$ was measured using Fura-2 microfluorometry.

Expt.	Sham	Burn	Burn + anti-CINC
Basal	100 ± 9	139 ± 12	108 ± 7
fmlp	302 ± 6	472 ± 20	360 ± 30

The burn injury clearly induced elevations in cytosolic $[Ca^{2+}]$ responses both in the absence (basal) and presence of fmlp. The pretreatment of rats with anti-CINC (5 mg/kg) resulted in an abrogation of burn-induced enhancement in $[Ca^{2+}]$ responses under basal and fmlp-stimulated conditions. These results suggest that neutrophil Ca^{2+} up-regulation occurring during burn injury is mediated to a large extent through endogenous CINC. (Support: NIH grants GM53235 and GM56865)

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NEUTROPHIL DEPLETION PREVENTS BURN INDUCED INTESTINAL VASCULAR PERMEABILITY ALTERATIONS IN RATS
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The present study evaluated burn-induced vascular permeability alterations of rat small intestine in vivo, and assessed the effect of neutrophil depletion in burn-injured rats on the altered intestinal microvascular permeability. ^{125}I-labeled bovine serum albumin (^{125}I-BSA) was injected intravenously and its leakage from circulation into intestinal tissue was determined by measuring tissue counts of ^{125}I-BSA.

Compared to sham, vascular albumin permeability increased 1.7-fold on day-1 post-burn and 3.0-fold on day-3 post-burn in ileum. In the jejunum, albumin permeability increased 1.8- and 2.5-fold on day-1 and day-3 post-burn, respectively. Intestinal tissue edema, determined as increases in tissue water contents, was noted in both intestinal segments on day-1 post-burn; no further increase in edema was found on day-3 post-burn. Neutrophil depletion prior to burn injury prevented the vascular leakage of albumin as well as edema in the ileum and jejunum on day-1 post-burn. On day 3 post-burn, the effect of prior neutrophil depletion on vascular permeability was less marked, and edema formation was not affected at all. These findings indicate that an absence of neutrophil prevents the loss of intestinal vascular barrier properties only in the initial periods after burns. (Support: NIH grants GM53235 and GM56865)

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STIFF NEUTROPHILS INDUCED BY G-CSF ATTENUATE INFLAMMATORY RESPONSE IN SEPTIC PATIENTS.

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We previously reported that intravenous administration of G-CSF decreased neutrophil deformability in anesthetized rats. In this study the effects of G-CSF on the blood rheology and neutrophil deformability were examined in septic patients with neutropenia. 5 patients in whom the total leukocyte count decreased below 6000/mm^3 and serum CRP concentration increased above 10 mg/dl were enrolled. Human recombinant G-CSF (2 μ g/ kg) was administered subcutaneously for 5 days. The whole blood samples were collected before G-CSF (day0) and after G-CSF (day5). By using a novel microchannel array etched on the single-crystal silicon tip which is simulated the microvasculature, the alterations of neutrophil deformability were observed under a microscope attached with a video camera. The number of obstructed microchannels (NOM) by stiff neutrophils was counted in a microscopic field. The time taken for 100 µl of whole blood to pass through the microchannel was determined. The total leukocyte count after G-CSF increased significantly compared with before G-CSF (day0: 4620 ± 975 vs. day5: 14666 ± 4698 /mm^3, mean ± SD, $p<0.01$). Serum CRP concentration after G-CSF was significantly decreased (day0: 13.7 ± 3.2 vs. day5: 7.6 ± 1.6 mg/dl, $p<0.02$). Increased adhesiveness and stiffening of neutrophils were observed after G-CSF. NOM after G-CSF was significantly larger than before G-CSF (day0: 3 ± 2 vs. day5: 12 ± 3 /field, $p<0.01$). Many microchannels were obstructed by neutrophils decreased their deformability, which contributed to the prolonged whole blood transit time. The whole blood transit time after G-CSF increased significantly compared with before G-CSF (day0: 37.3 ± 4.8 vs. day5: 136.3 ± 61.2 sec /100µl, $p<0.01$). These findings suggest that stiff neutrophils induced by G-CSF are involved in the reduction of the inflammatory response.

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DEXTRAN AND HESPAN RESUSCITATION CAUSES NEUTROPHIL ACTIVATION IN SWINE AFTER HEMORRHAGIC SHOCK.

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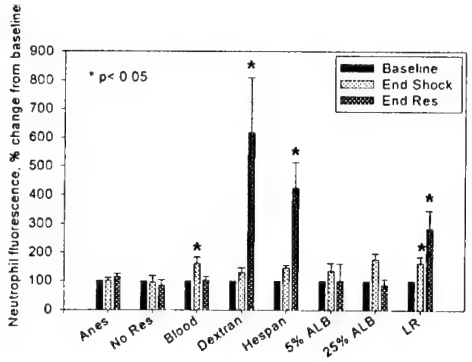
Activated neutrophils play a pivotal role in resuscitation injury. Our hypothesis was that the neutrophil activation depends on the type of resuscitation fluid and that artificial colloids would activate neutrophils more than natural colloids. **Methods:** 45 female swine (45-60kg) were subjected to a 28ml/Kg hemorrhage over 15 min, kept in shock for 1 hour, and then resuscitated for 1 hour as follows: 1) anesthesia only (n=5); 2) hemorrhage, no resuscitation (n=5); 3) whole blood -1x blood loss (n=6); 4) Dextran 40-1x blood loss (n=6); 5) 6% Hetastarch-1x blood loss (n=6); 6) 5% albumin-1x blood loss (n=6); 7) 25% albumin- 1/5x blood loss (n=6); 8) LR- 3x blood loss (n=6). Neutrophil oxidative burst activity was determined using a whole blood flow cytometery assay. **Results:** Animals resuscitated with Dextran, Hetastarch, and LR had significantly higher neutrophil burst activity. While the animals in the other groups showed no significant increase. **Conclusion**: Artificial colloids and LR (but not blood and albumin) cause significant neutrophil activation in swine following shock/resuscitation.



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PMN CHEMOTAXIS IS REGULATED BY FACTORS SECRETED BY IL-8- AND TNF-α-STIMULATED PMN.

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Purpose: We have shown that media conditioned by IL-1ß-, IL-8-, and TNF-α-stimulated PMN (CM-IL1ß, CM-IL8, and CM-TNF, respectively) affect PMN function and viability. Therefore, the purpose of this study was to examine whether PMN could also secrete factors which would promote and/or inhibit the recruitment of additional PMN to an inflammatory site. Methods: To test promotion of migration, CM ± fMLP were placed in the lower chamber of 24-well plates equipped with Transwell inserts. To test inhibition of migration, PMN were resuspended in CM for 0-1 hr and then placed into the Transwell filter with fMLP in HBSS

in the bottom chamber. For all experiments, PMN were allowed to migrate 2 hrs and the number of migrated cells was calculated. Results: CM-IL1ß had no effect on the migration of PMN in all conditions tested. CM-IL8 prepared by conditioning media for 4 hours contained pro-chemotactic factors which could not be blocked by IL-8 antibody and required active protein synthesis and secretion during conditioning. CM-TNF possessed strong anti-chemotactic activity, inhibiting PMN chemotaxis >50%. This activity is a result of intracellular changes as PMN incubated in CM-TNF for 1 hour demonstrated reduced chemotaxis when CM-TNF is removed prior to plating. Conclusions: We have shown that IL-8 and TNF-α induce PMN to secrete proteins which further amplify their pro- and anti-inflammatory effects by promoting and inhibiting, respectively, the migration of additional PMN into an inflammatory site.

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BURN-INDUCED INTESTINAL MUCOSAL HYPERPERMEABILITY IS NOT SEEN IN NEUTROPHIL-DEPLETED BURN-INJURED RATS.

O. Sir, F. Haque*, D. Fareed*, N. Fazal*, M. A. Choudhry, and M. M. Sayeed, Trauma/Critical Care Research Labs, Loyola University Chicago Medical School, Maywood, IL 60153

The mechanism of burn-induced increase in intestinal permeability to solutes which can pass through inter-epithelial cell spaces is not well understood. Inflammatory conditions in the bowel subsequent to burn injury could lead to infiltration of activated neutrophils into the intestinal wall. Oxidants released from such neutrophils could in turn damage intestinal epithelial integrity. We carried out experiments in sham and burn (25% TBSA skin scald) rats to investigate interepithelial cell passage of solutes, lactuloses and mannitol. These measurements were also made in a group of burn rats which were depleted of neutrophils via administration of a neutrophil antibody. ^{3}H-lactulose or ^{14}C-mannitol were infused into ileal segments and their passage into blood monitored as a function of time. Changes in the solute concentration (μM) per min were:

	Sham	Burn	PMN-depleted burn
Lactulose	100±20	420±30	130±10
Mannitol	90±20	270±30	90±10

The data show neutrophil depletion in burn injured rats prevent the increase in the permeability to both lactulose and mannitol in the ileum. These findings suggest that burn injury induced alterations in intestinal permeability result from accumulation of neutrophils and the accompanying release of oxidants in the intestinal wall. (Support: NIH grants GM53235 and GM56865)

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NO FORMATION AFTER INTESTINAL ISCHEMIA/REPERFUSION IN RATS: NOS-INDEPENDENT PATHWAY(S).

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To determine the sites and pathways of NO formation in the gastrointestinal tract we used electron paramagnetic resonance spectroscopy and the NO-trap diethyldithiocarbamate-Fe (DETC-Fe) to detect directly heme/non-heme iron nitrosyl complexes formed in blood, intestine, liver, and lung of rats subjected to local intestinal (SMAO 60 min) ischemia/reperfusion (I/R), in presence or absence of NOS inhibitors. Nitrosyl hemoglobin (Hb-NO) concentrations in circulating blood were significantly increased during ischemia and reperfusion. Hb-NO complexes were detected in ischemic intestine but not in normoxic lung and liver or reperfused intestine. In contrast to remote - organs lung and liver (no change), intestinal I/R resulted in an increase in NO/DECT-Fe complexes in intestinal tissues. Administration of the non specific NOS inhibitor L-NMMA caused a decrease of I/R-independent basal NO levels in lung and liver but did not influence the I/R-induced increase in NO formation in the intestinal tissues or the formation of circulating nitrosyl complexes. In support of this, RT-PCR analyses showed iNOS m-RNA expression in the lung but not in the intestinal tissues. The data suggest, that 1) at the early phase after I/R-, NO formation occurs in the ischemic intestinal tissue but not in the distant normoxic organs, despite of high circulating Hb-NO levels, 2) that NO formation in ischemic intestine is most likely NOS-independent, while 3) basal NO formation in normoxic remote tissues is partially NOS-dependent.

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Endothelial Protective Effects of Idoxifene in the Rat Splanchnic Artery.

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Estrogen stimulates endothelial nitric oxide (NO) release and attenuates endothelial dysfunction (ED) after ischemia and reperfusion (I/R). However, estrogen increases the risk of breast cancer. Our study determined if idoxifene (ido), a selective estrogen receptor modulator (SERM), may stimulate NO release and attenuate ED. In splanchnic artery rings (SARs) from ovariectomized (Ovx) rats, ido resulted in a dose-dependent relaxation with a maximal vasorelaxation (MV) of 56.3±4.9% (1 μM). Addition of L-NAME completely blocked ido-induced vasorelaxation. *In vitro* incubation of SARs with TNFα for 2 hours reduced vasorelaxation to an endothelium-dependent vasodilator, ACh (MV: 73±3.7% vs. 95±2.9% pre-TNFα, $p<0.01$). Addition of 0.3 μM of ido with TNFα restored MV to ACh to 86±2.6%

($p<0.05$ vs. TNFα alone). SARs from Ovx rats subjected to 60 minutes of I and 180 minutes of R (or when MABP declined to 45 mmHg) receiving only vehicle showed a marked ED as evidenced by decreased vasorelaxation to ACh (MV: 52±4.3%, $p<0.001$), but a normal vasorelaxation to an endothelium-independent vasodilator, acidified $NaNO_2$. Pre-treatment with ido *in vivo* at a dose of 0.5mg/kg/day for 3 days had no significant effect on ED (MV: 60±4.3%, $p>0.05$). However, treatment with higher doses (1 or 2 mg/kg/day for 3 days) markedly attenuated ED (MV to ACh: 78.7±3.7% and 72.4±3.6%, respectively, $p<0.01$ vs. vehicle). Moreover, treatment with ido significantly attenuated serum TNFα accumulation (214±13 vs. 98±32 pg/ml, $p<0.01$) after I/R. Taken together, these results demonstrate that ido, a novel SERM without adverse effects on reproductive organs, is an endothelium-dependent vasodilator and exerts significant endothelial protective effects against TNFα- and I/R-induced ED. These results suggest that SERMs have therapeutic potential in tissue I/R, hypercholesterolemia and shock where ED plays an important role.

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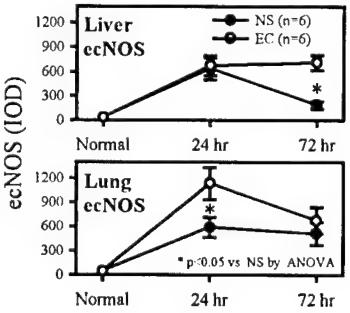
CONSTITUTIVE ENDOTHELIAL CELL NITRIC OXIDE SYNTHASE PROTEIN EXPRESSION DURING CHRONIC SEPSIS. AR Hatmaker*, PJ Matheson, RN Garrison, MB Carter*, MA Wilson. Ctr for Appl Microcirc Res & Dept of Surgery, Univ Lousiville & VAMC, Louisville, KY 40292.

INTRODUCTION: Nitric oxide (NO) derived from constitutive (endothelial cell) nitric oxide synthase (ecNOS) is thought to play an important role in the balance of vascular tone in resistance vessels. Patterns of gene expression and protein levels of ecNOS during chronic oxidative stress are not well described. We hypothesized that ecNOS protein levels remain unchanged following chronic bacteremia. METHODS: Male Sprague-Dawley rats underwent subcutaneous (back) sponge implantation 5-7 days prior to injection of either bacteria (EC, *E. coli* & *B. frag.* 10^9 CFU/mL saline each) or saline (NS, 2x 1mL). A second inoculation was made at 48 hr after the initial injection. At 24 or 72 hr after the initial inoculation, the animals were euthanized and liver and lung samples were harvested and processed for ecNOS westerns. RESULTS: ecNOS was elevated in both lung and liver compared to normals in both the EC and NS groups at 24 and 72 hr. EC elevated ecNOS at 24 hr in lung and at 72 hr in liver compared to NS. CONCLUSIONS: These data indicate increased ecNOS production in both the lung and liver in response to oxidative stress. Surgical stress and NS inoculation alone were sufficient to upregulate ecNOS protein expression at 24 & 72 hr, presumably altering vascular tone and flow distribution. Oxidative stress from EC increased ecNOS by different mechanisms and peaked earlier in lung than liver. (Supported in part by VAMR and NIH Grant T35 GM08561 funding.)



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Exogeneous substitution of NO via S-Nitroso-Human-Serum Albumin (S-NO-HAS) prevents microcirculatory alterations after hemorrhagic shock in the rat

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Arginin depletion during ischemia/reperfusion is reported to convert NO-synthase into an oxygen-radical (OR) generating enzyme. OR´s, induce inflammatory alterations of the hepatic microcirculation, e.g. narrowing of sinusoids. The present study was designed to evaluate the exogenous substitution of NO by S-NO-HSA in the prevention of pathophysiological alterations and organ damage after hemorrhagic shock. **Methods:** After approval of the ethics committee, Sprague Dawley rats were anesthetized and prepared for invasive hemodynamic monitoring and subjected to hemorrhagic shock for 60 min. Two groups were treated either with S-NO-HSA (10µmol/kg) or native HSA given by infusion for 60 min., during the first of three hours of resuscitation. Investigation of the hepatic microcirculation was started 5 hours after shock using intravital epifluorescence microscopy. Results were compared with a sham-control group (n=8 per groups). **Results:** After starting the S-NO-HSA infusion, MAP was significantly decreased during infusion, but completely restored afterwards. At 5 hours after shock, intravital microscopy showed in the shock group treated with HSA a significant narrowing of sinusoids in an area 90 µm around the central vein compared to sham group (HSA: 9,3µm; SEM: 0,1; Sham: 11,3µm, SEM: 0,2). Administration of S-NO-HSA prevented the decrease of sinusoidal diameters in comparison to HSA treatment $p<0,05$ (S-NO-HSA: 12,0µm, SEM: 0,2). **Discussion:** The results of the present study indicate a beneficial effect of exogeneously given S-NO-HSA in the prevention of shock induced pathological microcirculatory alterations in the liver. This effect might possibly attenuate organ damage after severe hemorrhagic shock.

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OXYGEN AS A RATE LIMITING SUBSTRATE FOR NITRIC OXIDE PRODUCTION BY iNOS IN CULTURED MACROPHAGES

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INTRODUCTION: Macrophages (MΦ) live in an environment of between 5 and 80 Torr O_2 *in vivo*. Oxygen is a substrate for MΦ NO production by inducible nitric oxide synthase (iNOS). We have previously reported that NO synthesis decreases with decreasing culture PO_2. We investigated the role of substrate limitation in this hypoxia-induced decrease in NO production. **METHODS:** RAW 264.7 cells (a mouse MΦ cell line) were cultured in constant, controlled oxygen tensions (PO_2 1, 8, 24, 40, 80, 150, 356, and 677 torr). Cells were stimulated with 0.1 µg/ml LPS and 100 U/ml interferon-γ. NO production was determined by the Greiss reaction. Cell lysate iNOS

activity was determined by the conversion of ^{14}C-Arginine to ^{14}C-Citrulline. Nitrite production and iNOS activity were normalized to protein concentration. The oxygen gradient in the culture media was measured using a fiberoptic, phosphorescence-quenching oxygen sensor. **RESULTS:** For PO_2 greater than 80 Torr, NO production was directly proportional to iNOS activity, and NO production scaled to iNOS activity had no dependence on culture PO_2. In contrast, for PO_2 between 8 and 80 Torr, scaled NO production varied with culture PO_2 in a sigmoid relationship, with an estimated K_m of 16 Torr PO_2 at the cell surface. **CONCLUSIONS**: The relationship between scaled NO production and culture PO_2 within the physiologic range of PO_2 suggests that cellular PO_2 regulates NO production via substrate dependence. The results suggest a connection between microcirculatory dysfunction and the inflammatory response in SIRS.
Supported by American Heart Association-SE PA Affiliate.

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CALCITONIN GENE-RELATED PEPTIDE ENHANCED NITRIC OXIDE RELEASE AND INDUCIBLE NOS ACTIVITY AND mRNA EXPRESSION IN LPS STIMULATED MOUSE PERITONEAL MACROPHAGES. X. Wang, J. Liu* and M. Chen*, Inst. of Vascular Med., Third Hospital, Beijing Med. Univ., Beijing, 100083, P. R. of China.

Previously we have showed that CGRP, a neuropeptide increases LPS-induced nitric oxide production in mouse peritoneal macrophages by using Griess method. In this study we further examined whether CGRP could modulate iNOS protein and mRNA expression from mouse peritoneal macrophages. Macrophages were obtained from the peritoneal exude of male Balb/c mouse. The cells were plated on culture dishes at a density of $5x10^5$ cells per well and allowed to adhere for 2 h. After incubation for 24 h, the macrophages were cultured with LPS 0.1 μg/ml and with or without CGRP (1 - 1000 nM) for 24 h. The results showed that CGRP enhanced 0.1 μg/ml LPS-induced NO release in a concentration-dependent manner. The NO production was increased from 2.3 ± 0.33 to a highest level of 4.17 ± 0.81 μM in $5x10^5$ cells by CGRP 10 nM. The cGMP level in macrophages was augmented when CGRP was added with LPS. However, CGRP had no direct effect on NO and cGMP production. CGRP increased the expression of inducible NOS protein in LPS-stimulated macrophages shown by immunocytochemistry method. The activity of iNOS was also enhanced by CGRP as compared with LPS-stimulated alone by detecting the ^{3}H-L-citruline formation from ^{3}H-L-arginine. We found by using RT-PCR method that CGRP also increased the LPS-induced iNOS mRNA levels. These data suggest that CGRP enhances LPS-induced NO release, iNOS activity and iNOS mRNA in mouse peritoneal macrophages.

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Protective effects of M40401 a superoxide dismutase mimetic in a rat model of splanchnic artery occlusion and reperfusion

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Splanchnic artery occlusion shock (SAO) causes an enhanced formation of reactive oxygen species (ROS), which contribute to the pathophysiology of shock. Here we have investigated the effects of M40401, a new *S,S*-dimethyl substituted biscyclohexylpyridine Mn-based superoxide dismutase mimetic (SODm, $k_{cat}=1.2 \times 10^{+9}$ M^{-1} s^{-1}), in rats subjected to SAO shock. Treatment of rats with M40401 (applied at 0.25, 2,5 or 25 μg/kg, 15 min prior to reperfusion), attenuated the mean arterial blood and the migration of polymorphonuclear cells (PMNs) caused by SAO-shock. M40401 also attenuated the ileum injury (histology) as well as the increase in the tissue levels of myeloperoxidase (MPO) and malondialdehyde (MDA) caused by SAO shock in the ileum. Immunohistochemical analysis for nitrotyrosine and for poly (ADP-ribose) synthetase (PARS) revealed a positive staining in ileum from SAO-shocked rats. The degree of staining for nitrotyrosine and PARS were markedly reduced in tissue sections obtained from SAO-shocked rats which had received M40401. Reperfused ileum tissue sections from SAO-shocked rats showed positive staining for P-selectin and for anti-intercellular adhesion molecule (ICAM-1) in the vascular endothelial cells. M40401 treatment markedly reduced the intensity and degree of P-selectin and ICAM-1 in tissue section from SAO-shocked rats. M40401 treatment significantly improved survival. Additionally, the very high catalytic activity of this new mimetic (comparable to the native human Cu/Zn SOD enzyme and exceeding the activity of the human Mn SOD enzyme) translates into a very low dose (~μg/kg) required to afford protection in this SAO model of ischemia reperfusion injury. Taken together, our results clearly demonstrate that M40401 treatment exerts a protective effect and part of this effect may be due to inhibition of the expression of adhesion molecules and peroxynitrite-related pathways with subsequent reduction of neutrophil-mediated cellular injury.

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Protective effect of Tempol, a membrane-permeable radical scavenger, on the multiple organ failure induced by zymosan in the rat

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Here we have investigated the effects of tempol, a membrane-permeable radical scavenger, on the multiple organ failure (MOF) caused by zymosan in the rat. Zymosan administration causes an enhanced formation of reactive oxygen species (ROS), which contribute to the pathophysiology of MOF. MOF was induced by zymosan (500 mg/kg, suspended in saline solution, i.p.). After zymosan or saline administration, animals were monitored for wsssssssssvgbffgxxevaluation of loss of body weight and

mortality for 12 days. Treatment of rats with tempol (10, 30 or 100 mg/kg intraperitoneally, 1 and 6 hour after zymosan) attenuated the peritoneal exudation and the migration of polymorphonuclear cells (PMNs) caused by zymosan in a dose-dependent fashion. Tempol also attenuated the lung, liver and intestine injury (histology) as well as the increase in the levels of myeloperoxidase (MPO) and malondialdehyde (MDA) caused by zymosan in the lung, liver and intestine. Immunohistochemical analysis for nitrotyrosine and for poly (ADP-ribose) synthetase (PARS) revealed a positive staining in lung, liver and intestine from zymosan-treated rats. The degree of staining for nitrotyrosine and PARS were markedly reduced in tissue sections obtained from zymosan-treated rats, which had received tempol (100 mg/kg i.p.). This study provides the first evidence that tempol, a small molecule that permeates biological membranes and scavenges ROS, attenuates the degree of MOF associated with zymosan-induced peritonitis in the rat.

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DEXTRAN CONJUGATION OF POLYNITROXYLATED αα-CROSSLINKED HEMOGLOBIN IMPROVES HEMODYNAMICS AND OUTCOME OF HEMORRHAGED RATS. P. Buehler, L. Ma*Ŧ, C.E. Trimble*Ŧ, C.J.C. Hsia*Ŧ and A. Gulati*. University of Illinois, Chicago, IL 60612 and Ŧ Synzyme Technologies LLC, Irvine, CA 92618

Objective: Our previous studies indicate polynitroxylation (PN) attenuates the hypertensive response associated with αα-crosslinked Hb (ααHb) (SHOCK 11:S1, A54). However, the improvements in systemic and regional hemodynamics did not lead to significant improvement in outcome (base deficit and survival). Therefore, we hypothesize that increasing the molecular weight of PN-ααHb with dextran surface conjugation may improve outcome in our rat model of shock resuscitation. Methods: Dextran (Dex) conjugated PN-ααHb was prepared by decorating the surface of the hemoglobin protein with hetastarch to create an HBOC with a MW of approximately 240 kd. Anesthetized rats (350-400 g) were hemorrhaged and then resuscitated with 100% shed blood volume of (I) ααHb or (II) Dex-PN-ααHb. The following parameters were measured at baseline, hemorrhage, 30 and 60 min following resuscitation: mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR), base deficit (BD) and regional blood flows (radioactive microsphere technique). Results: During hemorrhage MAP was maintained at 35 to 40 mmHg for 30 min. Volume of blood withdrawn (mls) for the ααHb group and Dex-PN-ααHb group were 9.88±0.12 and 9.89±0.4 respectively. The following table shows hemodynamic parameters at 30 and 60 minutes post resuscitation (Mean ± SEM): (*indicates a significant difference between groups $p < 0.05$, determined by Student's t-test, n=8 for each group)

	MAP mmHg		CO mL/min.		TPR mmHg/mL/min.	
Group	30 min	60 min	30 min	60 min	30 min	60 min
(I)	115 ± 3	118 ± 4	157± 11	97 ± 6	762± 59	1257 ± 89
(II)	87 ± 4 *	88 ± 3 *	195±14*	155±8*	461±41*	580±41*

Organ blood flows were significantly improved in the kidneys, GIT and musculoskeletal system at 30 and 60 minutes with Dex-PN-ααHb resuscitation. BD (mmol/L) values for ααHb and Dex-PN-ααHb were -15±1 and -14±0.9 respectively after hemorrhage. 60 min post infusion Dex-PN-ααHb improved BD to -4.7± 0.5* compared to - 8.6±1 with ααHb. Survival times (min) were as follows; ααHb, 404 ± 61; Dex-PN-ααHb, 940 ± 140*. Conclusion: Dex-PN-ααHb did not produce the pressor response observed with ααHb, it also improves systemic hemodynamics and regional circulation. Furthermore, improvements in base deficit and survival time associated with Dex-PN-ααHb resuscitation may be attributed to decreased vascular extravasation and increased duration of action.

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EFFECT OF A CASPASE-1 INHIBITOR ON MORTALITY AND SERUM IL-1β AND IL-6 LEVELS IN IN A RAT MODEL OF ENDOTOXEMIA

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Objective: To investigate whether the caspase inhibitor Ac-YVAD-CYK can lower mortality and modify levels of IL-1β and IL-6 in a rat model of endotoxemia.

Design: Randomized controlled animal study in rats.

Methods: 20 rats were randomly assigned to receive either 25 µmol/kg of body weight Ac-YVAD-CYK or drug vehicle prior to the bolus administration of i.v. endotoxin (65 mg/kg body weight of S. enteritidis LPS (LD_{80})). The animals were monitored 8 hours for mortality and at time points 0, 1, 2, 4 and 8 hours following LPS administration, serum samples were withdrawn and cytokine levels determined by ELISA.

Results: Mortality tended to be lower in the caspase–1 inhibition group (4/10) than in the control group (8/10) ($p = 0.0566$). There was no difference in IL-1β and IL-6 levels between the both groups. e.g. at the peak IL-1β were 930 pg/ml (± 301 pg/ml) in the control and 1060 pg/ml (± 487 pg/ml) in the treated group and IL-6 was 35835 pg/ml (± 301 pg/ml) in the control and 35440 pg/ml (± 2058 pg/ml) in the treated group.

Conclusion: Preliminary studies showed a trend for decreased LPS induced mortality in rats pretreated with the caspase-1 inhibitor. This effect appeared to be independent of changes in IL-1β or IL-6 serum levels.

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EFFECT OF INDOMETHACIN ON THE RESUSCITATIVE ACTIONS OF DIASPIRIN CROSS-LINKED HEMOGLOBIN (DCLHb) IN HEMORRHAGED RATS. S. Mehendale*, B. Saini*, P. Buehler, R. Palaparthy* and A. Gulati*. University of Illinois at Chicago, IL 60612

DCLHb, a 64.5kD hemoglobin based oxygen carrier, was found to be an effective resuscitative agent in animal models. However, it produced serious adverse effects in phase III clinical trials which may be due to the significant pressor effect of DCLHb. Modulation of DCLHb's pressor effect and resuscitative action due to concurrent administration of vasoactive agents, involving NO, endothelin and adrenergic mechanisms, has been studied. However, the role of prostaglandins (PG) in modifying cardiovascular response to DCLHb has not been studied. The present study investigates the effect of indomethacin (PG inhibitor) on the resuscitative actions of DCLHb in hemorrhaged rats. Methods: Urethane-anesthetized rats were hemorrhaged at a rate of 1 ml/min to a mean arterial pressure (MAP) of 35-40 mmHg. At 30 min. of hemorrhage, rats were resuscitated using 100 % shed blood volume replacement with either (i) vehicle: Ringer Lactate+Propylene Glycol (0.8ml/kg; Grp I), (ii) DCLHb (10 %, Grp II) or (iii) indomethacin (5 mg/kg, iv) + DCLHb (Grp III). MAP, cardiac output (CO), total peripheral resistance (TPR), base deficit (BD) & regional blood flow (radioactive microsphere technique) were measured at baseline, after hemorrhage, at 30 min & 60 min following resuscitation. Results: The table shows physiological parameters measured at 60 min post-resuscitation (% change from hemorrhage, * indicates difference from hemorrhage, # indicates difference between Grp II and Grp III; $p<0.05$; n=6/group).

Group	MAP	CO	TPR	BD
Grp I	14 %	-11 %	39 %	-5.6 %
Grp II	215 % *	314 %*	-14 %	79 %*#
Grp III	154 % *	277 %*	-21 %	31.9 %*

Regional blood flow significantly decreased in Grp I, in all tissues except brain, compared to baseline. In Grp II, perfusion to skeletal muscle, GIT & kidney increased at 30 min. & was restored to baseline at 60 min. In Grp III, blood flow increased only to heart at 30 min while renal & skeletal muscle perfusion significantly decreased at 60 min. Conclusions: Indomethacin attenuated DCLHb-induced improvement in perfusion to the kidney, GIT & skeletal muscle; & decreased the recovery of BD. Systemic hemodynamc parameters were minimally affected. Indomethacin, a non-specific cyclooxygenase blocker, may affect both vasoconstrictor & vasodilator PG; therefore some responses to DCLHb are attenuated while others are not. We conclude that prostaglandin mechanisms may play a role in DCLHb-induced increase in tissue perfusion.

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MODULATION OF FREE RADICAL INJURY BY PHARMACOLOGICAL AGENTS IN HEMORRHAGE MODEL IN RATS. P. Seth*, R. Kumari*, G.S. Sidhu*, R.K. Maheshwari,* (Spon : F. Rollwagen).
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Resuscitation from hemorrhagic shock initiates profound changes in many physiologic processes including alterations in the redox state of the environment. These changes are prominent in liver and are likely to contribute to end organ damage and resultant dysfunction after shock. Research in this area have substantially indicated towards the potential of free radical scavenging strategy for better management of the pathophysiology following hemorrhage-resuscitation (H/R) injury. We studied the effect of novel pharmacological agents picroliv and curcumin, the two plants products, on the free radical scavenging enzyme system and nitric oxide release in a H/R model in adult rats. Anesthetized rats were subjected to hemorrhagic shock by bleeding them 30 ml/kg body weight. After 60 minutes of shock rats were resuscitated twice the bleed out volume, with Ringer's lactate solution and sacrificed 2 hrs after resuscitation. We observed that lipid peroxidation and nitric oxide release was increased following H/R injury. Both picroliv and curcumin pre-treatments resulted in attenuation of lipid peroxidation and NO release following H/R. We also observed that the test agents altered the glutathione contents, activity of glutathione peroxidase and glutathione reductase in a favorable manner, thereby suggesting better antioxidant status. These findings suggest that these plant products have the potential to be developed as protective agents against H/R injury. Work supported by Office of Naval Research Grant (G174HV).

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POLYNITROXYL-STARCH (PNS) PLUS TEMPOL IN COMBINATION WITH A HEMOGLOBIN BASED OXYGEN CARRIER (HBOC) IMPROVES HEMODYNAMICS OF HEMORRHAGED RATS. H. Wang*, P. Buehler, S. Mehendale*, L. Ma*Ŧ, C.E. Trimble*Ŧ, C.J.C. Hsia*Ŧ and A. Gulati*. University of Illinois, Chicago, Il 60612 and Ŧ Synzyme Technologies LLC, Irvine CA 92618.

Objective: Preliminary studies indicate that PNS plus the antioxidant TEMPOL reduces hemoglobin toxicity. TEMPOL in addition to having antioxidant action is a potent vasodilator and may attenuate the pressor response associated with certain HBOCs. Therefore, we hypothesize that the addition of PNS plus TEMPOL to a solution containing an HBOC may improve systemic and regional circulation compared to donor blood when both are given as 100% shed blood volume. Methods: The following two groups were evaluated; (I) 70% 240 kd MW HBOC (from a 10% solution) + 30% PNS (from a 5% solution) + 6 mg/mL TEMPOL and (II) Whole blood, from donor rats bled via the abdominal aorta and stored in (CPDA -1) USP at 4° C for 1 to 3 days before use (American Red Cross 1751, Jan 1999). In anesthetized rats, the following were measured after 30 min hemorrhagic shock (mean arterial pressure maintained at 35-40 mmHg) and 60 min 100% volume resuscitation with one of the two treatments: mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR) and regional blood flows (radioactive microsphere method). Results: The table shows hemodynamic parameters at 30 and 60 min post resuscitation (Mean ± SEM, * indicates a significant difference $p<0.05$ from whole blood values, determined by Student's t-test). Group (I) n=9 and Group (II) n=7.

	MAP mmHg		CO mL/min/kg		TPR mmHg/mL/min/kg	
Group	30 min	60 min	30 min	60 min	30 min	60 min
(I)	84 ± 4 *	95 ± 3	432±50*	229±23*	212±20*	469±66*
(II)	97 ± 3	90 ± 5	173±22	117±13	584±83	773±26

Organ blood flow significantly improved to the brain, GIT and pancreas at 30 min and to the heart, kidneys and musculoskeletal system at both 30 and 60 min post-resuscitation in group (I) compared to group (II) (whole blood). Conclusion: The addition of PNS plus TEMPOL to a high molecular weight HBOC appears to transiently reduce MAP over the initial 30 min following infusion while reducing TPR and increasing CO. Over the next 30 minutes the effects of PNS plus TEMPOL on MAP appear to subside while TPR remains significantly lower and CO significantly higher than that which is seen with whole blood resuscitation.

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NEUROPROTECTIVE EFFECTS OF HYPERTHERMIC PRECONDITIONING AFTER FOCAL CEREBRAL ISCHEMIA IN RATS: A ROLE OF ADENOSINE RECEPTORS

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Hyperthermic preconditioning may have neuronal protections in whole brain ischemia. We have now examined the effects of hyperthermic pretreatment on infarcted volume after middle cerebral artery occlusion (MCAO) in rats. Group assignment: 1) sham-control (n=8): keeping at normothermia (37±0.2C° in pericranial temperature) for 15 min under isoflurane anesthesia, then waiting in awake state for 0.5, 3, 6, 18, 24, 48 hours before 2 hour focal cerebral ischemia; 2) hyperthermia group (n=8): subjecting to 42±0.5C° for 15 min under anesthesia, then received the same treatment as the sham. ANOVA with post hoc test, Dunnet's test, X^2-test were used as appropriate ($p<0.05$). Infarcted volume in hyperthermic animals of 18, 24 hour waiting time were significantly smaller than the sham control, but not in rats of the other waiting

hours. DPCPX, a selective A1 adenosine receptor antagonist, partially reversed the reduced effects by hyperthermic preconditioning on the infarcted volume after focal ischemia, whereas the agent itself did not affect the volume after ischemia. These data suggest that hyperthermic pretreatment has reduced effects on MCAO induced cerebral infarction, possibly via partial mediation of adenosine receptors in the brain.

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Systemic Complement Depletion Does Not Improve Cardiac Performance Following Hemorrhagic Shock & Resuscitation.
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Complement activation may occur during hemorrhagic shock and resuscitation and contribute to cardiac dysfunction similar to *in vitro* and *in vivo* myocardial ischemia-reperfusion injury models. We examined hemodynamic stability and cardiac performance in a model of hemorrhagic shock and resuscitation after systemic complement depletion (CD). Methods: A fixed pressure model of hemorrhage was performed on New Zealand White rabbits to a mean arterial pressure (MAP) of 35-40 mm Hg. After a 3-hour resuscitation period, rabbit hearts were placed on a Langendorff apparatus. Cardiac performance was gauged by coronary perfusion pressure (CPP), left ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP). Cardiac tissue was subjected to histologic analysis by either propidium iodide (PI) staining to assess cell viability, or immunohistochemistry for the membrane attack complex (MAC). In some animals, complement was depleted using serial cobra venom factor i.p. injections prior to study. Results: Removal of a larger blood volume was possible in the CD (63.7 ± 4.9 ml) vs. hemorrhage group (50.9 ± 9.6 ml), $p = 0.0002$. There was no significant difference in MAP in the CD vs. hemorrhage group, nor was a significant difference found between the hemorrhage, CD and control groups in regard to CPP and LVEDP. Hemorrhage resulted in a significantly lower LVDP (49.3 ± 15.5 mm Hg) vs. control (78.6 ± 14.6 mm Hg) $p < 0.05$, which was not improved by CD (55.4 ± 13.7 mm Hg). PI uptake was greater in the hemorrhage group (1517 ± 598 $OD_{530/620}$/g) vs. control (652 ± 142 $OD_{530/620}$/g), $p = 0.008$, but not compared to the CD group (1092 ± 524 $OD_{530/620}$/g), $p = 0.11$. Initial immunohistochemistry for both hemorrhage and CD groups revealed myocytes with MAC. Conclusion: Changes in cardiac function induced by hemorrhagic shock and resuscitation are associated with histological evidence of myocardial injury and complement activation, however, systemic complement depletion in advance does not improve cardiac function, and complement activation may still occur.

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HYPOXANTHINE AND OTHER PURINES INHIBIT THE ACTIVATION OF POLY(ADP-RIBOSE) SYNTHETASE: IMPLICATIONS FOR THE PATHOPHYSIOLOGY OF ISCHEMIA-REPERFUSION INJURY
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The release and extracellular degradation of purines has previously been implicated in the pathophysiology of various ischemic conditions. Ischemic conditions are associated with activation of the nuclear enzyme poly (ADP-ribose) synthetase. Therefore, in *in vitro* studies, we have tested the possibility that adenosine and its degradation products affect PARS activation. In cultured RAW cells, peroxynitrite induced a marked increase in the activity of PARS, which was dose-dependently inhibited by hypoxanthine and inosine (100 μM- 3 mM). Adenosine, at 3 mM, exhibited a slight inhibitory effect on PARS. The difference between the reference compound (the prototypical PARS inhibitor nicotinamide) and the most potent purine tested (hypoxanthine), was rather small (EC_{50} values approx. 1 and 2 mM, respectively). Because differential cell uptake of the purines and PARS inhibitors may influence the potency of PARS inhibition, we also tested the effect of the purines and reference compounds in a cell-free PARS assay. Again, the potency of the purines was hypoxanthine > inosine > adenosine, with nicotinamide and 3-aminobenzamide becoming significantly potent relative to the purines. These results indicate that limited cell uptake markedly reduces the PARS inhibitory potency of 3-aminobenzamide and nicotinamide, but is not a significant limiting factor for the purines. Hypoxanthine (potently), and the other purines tested (weakly) inhibited the production of nitrite/nitrate in response to immunostimulation. These data are all consistent with a cytoprotective effect of certain purines, which is mediated via their inhibitory effect on PARS. We speculate that certain purines, when released during ischemia, may protect against the subsequent reperfusion injury by inhibition of PARS.

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SESQUITERPENE LACTONE PARTHENOLIDE EXERTS ANTIINFLAMMATORY EFFECTS IN MYOCARDIAL ISCHEMIA/REPERFUSION INJURY.
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Sesquiterpene lactones are extracts of common medicinal *asteracae* plants used in folk medicine for their anti-inflammatory activity. Although the mechanisms of action are not well understood, *in vitro* studies have shown that these compounds may interfere with gene regulation. This study examines the effects of parthenolide, a major lactone, in experimental myocardial ischemia and reperfusion. Myocardial injury was induced in male Wistar rats by 30 min occlusion of the left coronary artery followed by reperfusion for 2 hours. In vehicle-treated rats, ischemia and reperfusion caused myocardial injury, as evaluated by plasma levels of creatinphosphokinase and by histological examination. Elevated tissue levels of myeloperoxidase activity were indicative of a significant infiltration of neutrophils. This event paralleled the occurrence of oxidative and nitrosative damage, as evaluated by a marked increase in tissue malondialdehyde levels and an intense immunostaining for nitrotyrosine. These inflammatory events were preceded by nuclear translocation of the transcriptor factor NF-κB, with a maximum peak at 15 min after reperfusion. Administration of parthenolide (10 min before reperfusion) lowered myocardial necrosis and plasma creatinphosphokinase activity, reduced

neutrophil infiltration and the subsequent oxidative and nitrosative damage. These beneficial effects were associated with inhibition of nuclear translocation of NF-κB. The data suggest that parthenolide suppresses myocardial damage, at least in part, by regulating the early inflammatory response of reperfusion at the genetic level through inhibition of NF-κB.

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Endothelin B receptors modulate hepatic metabolic response in endotoxin primed rats. R Baveja, D Harding, Y Yokoyama, N Sonin, JX Zhang and MG Clemens. Department of Biology, Univ of North Carolina-Charlotte, NC 28223.

Endotoxin (LPS) increases sensitivity of liver microcirculation to endothelins with increased ET_B receptor proportion. Here, we report the hemodynamic and metabolic significance of ET_B receptors in normal and LPS (1mg/kg, ip for 24 hrs) primed rats. Portal pressure, O_2 consumption (VO_2) and glucose output were measured in an isolated liver perfusion. We also determined the role of NO and prostaglandin synthesis. In LPS, IRL-1620 (ET_B agonist) caused a similar increase in portal pressure as in sham (43.8% ± 3.1 sham; 51.3% ± 11.1 LPS at end of infusion). The peak portal pressure response was higher in presence of NOS inhibitor L-NAME, with a significant increase in sham compared to LPS (110% ±9.9 Sham; 85.8 %± 9.8 LPS). In the presence of indomethacin, portal pressure peak response was similar but less sustained in both groups. ET-1 (ET_A + ET_B agonist) produced a significant decrease in oxygen consumption and gluconeogenesis in controls while IRL-1620 did not change VO_2 or gluconeogenesis in either sham or endotoxin primed animals. However, in the presence of IRL 1620 plus L-NAME, VO_2 was significantly decreased. Gluconeogenesis mirrored the changes in VO_2. These results indicate that ET_B receptor stimulation prevented the decrease in VO_2 caused by ET_A receptor activation via an NO dependent pathway. In contrast, indomethacin pretreatment had opposite effects as L-NAME on the ET_B response. In isolated hepatocytes, both IRL-1620 and ET-1 caused a similar increase in VO_2 (6.9 ± 1.07 and 6.09 ± 0.44 ml/100 g body wt with ET-1 and IRL-1620 respectively) indicating that the effects of ET_A and ET_B receptors are mediated via non parenchymal cells. Thus, in spite of similar portal pressure response with ET-1 and IRL-1620, only the ET_B agonist maintains oxygen consumption and gluconeogenesis. The differential effects of ET-1 and ET_B agonists are dependent on both NO and cyclo oxygenase dependent pathways in nonparenchymal cells. Supported by DK38201

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INDUCIBLE NITRIC OXIDE SYNTHASE IS REQUIRED FOR ENTEROCYE APOPTOSIS AFTER HEMORRHAGIC SHOCK. EP Nadler*, VD Schuchert*, S Alber*, HR Ford. Children's Hospital of Pittsburgh, Pittsburgh PA, 15213

Introduction: Hemorrhagic shock may lead to gut barrier dysfunction and subsequent infectious complications. However, the mechanisms involved have not been defined. We have previously shown that endotoxic shock is associated with iNOS upregulation and enterocyte apoptosis. We hypothesized that iNOS induction in the intestine after hemorrhagic shock would lead to enterocyte apoptosis. **Methods:** Wild type (WT) or iNOS knockout (KO) mice were hemorrhaged to a mean arterial pressure of 25 mmHg for 2.5 h and then resuscitated. At 24 h, the terminal ileum was harvested for immunohistochemical detection of apoptosis using the TUNEL assay. Sham animals were cannulated but not hemorrhaged. **Results:** Sham operation did not increase the incidence of apoptosis in WT or KO mice. WT mice had a significant increase in enterocyte apoptosis 24 h after hemorrhage and resuscitation (Table). KO mice did not show an increase in apoptosis after shock. **Conclusions:** Upregulation of iNOS and NO production after hemorrhagic shock lead to enterocyte apoptosis. NO scavengers or iNOS inhibitors may be beneficial in preventing the gut-barrier dysfunction associated with hemorrhage and resuscitation.

Group	Villi Examined	Apoptotic Nuclei	Apoptotic Nuclei/villus
WT Sham (n=3)	42.0 ± 4.4	45.7 ± 36.0	1.04 ± 0.78
WT Shock (n=3)	47.0 ± 5.2	250.7 ± 93.4	5.24 ± 1.36*
KO Sham (n=2)	36.0 ± 15.6	49.0 ± 52.3	1.16 ± 0.95
KO Shock (n=3)	32.7 ± 4.0	46.7 ± 40.3	1.53 ± 1.46

*$p < 0.05$ versus WT sham, KO sham, and KO shock; Fisher's Least Significant Difference

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IRAK MEDIATES POSTBURN MYOCARDIAL CONTRACTILE DYSFUNCTION. J.A. Thomas*, J White*, J.W. Horton, UT Southwestern Med Ctr, Dallas, TX 75390.

Burns elicit a complex, multifaceted host protective response. Myocardial dysfunction, both systolic and diastolic, figures prominently in the reaction to this injury. Our previous studies have shown that burn trauma activates cardiac NF-κB and promotes TNF-α synthesis. Many different proximal signal transduction pathways converge to activate NF-κB, but little is known about which of these is activated after burn injury. We investigated the role of IRAK, a kinase that functions in the conserved Toll/IL-1 signaling module. We hypothesized that burn injury activates this pathway and specifically that IRAK-deficient animals would be resistant to burn-induced myocardial depression. IRAK knockout (KO) and wild-type (WT) mice were given a 3° scald burn over 40% TBSA and fluid resuscitated (IP). WT and KO shams were included for controls; 24 hrs postburn, hearts (N=5-8/group) were perfused (Langendorff). There were no significant differences in cardiac function between the WT and KO shams. Burn trauma in WT mice triggered cardiac systolic and diastolic dysfunction as indicated by lower developed LVP (mmHg), and diminished ±dP/dt max (mmHg/sec). IRAK-deficient mice displayed significant myocardial protection against this burn-induced myocardial dysfunction compared to WT burns. We conclude that IRAK specifically, and the Toll/IL-1 signaling cassette in general,

contribute to the myocardial dysfunction resulting from burn injury.

	WT Sham	IRAK KO Sham	WT Burn	IRAK KO Burn
LVP	113.2 ±5.4	101.8, ±5.1	57.2 ±1.7*	70.6 ±6.5
+dP/dt max	2580 ±97	2444 ±102	1400 ±32*	1725 ±138
-dP/dt max	2176 ±59	1973 ±126	996 ±43*	1317 ±141

**indicates significant difference, p<0.01*

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DEPRESSED TRAUMA PATIENT MØ IL-18 LEVELS LEAD TO DECREASED T CELL IL-13 LEVELS. C. Miller-Graziano, A. De, **P.Bankey.** UMASS Med. School, Worcester, MA 01655

Trauma patient monocytes (MØ) have reduced T cell activating potential partly due to decreased production of immunostimulatory (IL-12, IL-15) monokines. Post-trauma dysfunctional monokine production may extend to other immunostimulatory monokines. We've shown monokine participation as essential for maximal T cell IL-13 production and that T cells from immunosuppressed patients have reduced IL-13 production. Here, we assessed the ability of trauma [mechanical (ISS>15) or thermal (>30% TBSB)] patients' MØ culture supernates stimulated with MDP+SEB (20μg/ml+0.5μg/ml) to augment IL-13 production by allogeneic normal T cells. Stimulated control normal or immunocompetent trauma patients' MØ culture supernates significantly ($p<0.001$) augmented allogeneic normal T cell IL-13 levels. MØ culture supernates from immunosuppressed (50% depressed T cell proliferation) trauma patients failed to augment allogeneic normal T cell IL-13 levels, suggesting they had reduced production of monokines necessary to co-stimulate T cell IL-13. A newly described monokine, IL-18, enhances IL-13 production by T cells. Failure of immunosuppressed trauma patients' MØ culture supernates to augment allogeneic T cell IL-13 production could reflect depressed MØ IL-18 production. IL-18 levels assessed (Elisa) in MØ culture supernates from immunosuppressed patients were significantly ($p<0.01$) depressed as compared to normal control values and failed to induce any augmentation of IL-13 production by normal allogeneic T cells. This indicates depressed post-trauma MØ IL-18 production as one mechanism for failure of post-trauma MØ accessory/co-stimulatory functions on T cells.

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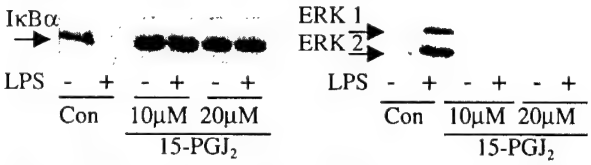
INHIBITION OF LPS-INDUCED ERK 1/2 ACTIVATION AND IκBα DEGRADATION BY 15-DEOXY-$\Delta^{12,14}$-PGJ_2. *MK Guyton, SH Ashton, GE Tempel, PV Halushka, and JA Cook. Medical University of South Carolina, Charleston, S.C. 29425.*

The prostaglandin metabolite, 15-deoxy- $\Delta^{12,14}$-prostaglandin J_2 (15-PGJ_2) of the PGJ_2 family possesses potent anti-inflammatory properties. Previous studies in our laboratory have demonstrated that nitric oxide, tumor necrosis factor-alpha, and thromboxane B_2 levels are suppressed in LPS-stimulated rat peritoneal macrophages (MØ) that have been treated with 15-PGJ_2. Although the effects of 15-PGJ_2 were initially thought to be through activation of the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPARγ), recent data suggest that 15-PGJ_2 may exhibit PPARγ-independent effects. Thus, we hypothesized that 15-PGJ_2 may be acting on cell signaling proteins upstream of PPARγ. LPS activates several distinct signaling pathways in MØ that lead to the production of pro-inflammatory mediators. These pathways include inhibitor kappa B-alpha (IκBα) degradation which leads to activation of nuclear factor κB (NFκB) and the mitogen-activated protein kinase cascade which leads to activation of the extracellular signal-regulated kinases (ERK 1/2). Therefore, to test the hypothesis that 15-PGJ_2 alters signaling, MØ were pretreated (1 hour) with 15-PGJ_2 and then stimulated for 30 minutes with LPS. Cells were collected to determine IκB degradation and ERK phospho-activation via western blotting of the proteins (data below).



Representative Western Blot Analysis (n=2) of the effects of 15-PGJ_2 on IκBα degradation and ERK 1/2 activation in Control (con) and LPS (50μg/ml)-treated MØ.

Pretreatment with 15-PGJ_2 inhibited IκBα degradation in LPS-induced MØ compared to groups treated with LPS alone. LPS-induced ERK 1/2 phospho-activation was also inhibited by 15-PGJ_2. The data suggest that the anti-inflammatory properties of 15-PGJ_2 may be mediated, in part, by inhibiting proximal LPS signaling pathways. Supported by NIH GM27673.

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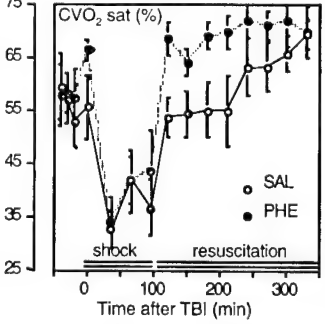
CEREBRAL PERFUSION PRESSURE (CPP) DIRECTED THERAPY AFTER TRAUMATIC BRAIN INJURY (TBI) AK Malhotra*, JB Schweitzer*, TC Fabian, KG Proctor Depts. of Surgery & Physiology, Univ. of Tenn. Health Sci. Ctr., Memphis and Dept. of Pathology, Quillen College of Med., Johnson City, Tenn.

Background: There are no Brain Trauma Foundation guidelines for CPP directed therapy due to insufficient level I or II evidence. The purpose of this study was to compare acute outcome with this management strategy. Methods: Anesthetized, ventilated (FiO_2=0.5) swine received TBI via cortical fluid percussion (6-8 ATM) followed by 45% hemorrhage. After 1 hr, all animals were resuscitated with saline equal to 3 times shed blood volume, followed by supplements for systolic blood pressure <100 mmHg or heart rate >100 beats/min. One group (PHE, n=7) received phenylephrine titrated to maintain CPP=80 mmHg, the other did not (SAL, n=4). Cerebral venous O_2 saturation (CVO_2 sat) was measured in the sagittal sinus. Results: ICP increased from 5 mmHg during shock, to 15-20 mmHg with resuscitation and was similar in both groups. MAP corrected from <55 mmHg to >85 mmHg with resuscitation. Over the next 4 hrs, MAP and CPP declined to 70±5 and 55±3 mmHg in SAL, despite supplements. In PHE, MAP and CPP were maintained at 95-100 and 75-85 mmHg. EEG, total fluids, heart rate, cardiac index, ABGs, serum electrolytes, glucose, and lactate were similar between groups. Before TBI, 5%CO_2 evoked a 14 ±2% increase in CVO_2 sat, indicating vasodilatation.



By 4 hrs, this response was entirely absent in SAL but was 14±4% in PHE. Conclusion: After TBI + hypotension, CPP directed therapy maintains brain oxygenation, and cerebrovascular CO_2 reactivity without increasing ICP. Supported by Office of Naval Research

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EFFECTS OF FLUID RESUSCITATION IN CEREBRAL INTRACELLULAR CALCIUM IN TRAUMATIC BRAIN INJURY ASSOCIATED WITH HEMORRHAGIC SHOCK. M Balbino*, LF Poli de Figueiredo, A Capone Neto, R Prist, AT Ferreira, M Rocha e Silva. Heart Institute-InCor, Univ. São Paulo Medical School, SP 05403-000, Brazil.

Background: Calcium is one of the triggers involved in ischemic neuronal death. The injury can be truly delayed, even after cells repolarize, and resume physiological and metabolic functions. Thus, it would be reasonable to prevent calcium influx in early stages of traumatic brain injury (TBI). Since hypotension is a strong predictor of outcome in TBI, we tested the hypothesis that fluid resuscitation blunts calcium influx in hemorrhagic shock associated to head injury. Methods: Fifteen ketamine-halotane anesthetized mongrel dogs (18,7 ± 1,4 kg). underwent unilateral cryogenic brain injury. Blood was shed in 5 min to a target mean arterial pressure (MAP) of 40-45 mmHg, maintained for 20 min (shed blood volume = 26±7 ml/kg). Animals were then randomized into three groups: HS (7.5% NaCl, 4 ml/kg, in 3 min), LR (Lactate Ringer's, 33 ml/kg, in 15 min) and CT (controls, no fluid resuscitation). Twenty min later, a craniotomy was undertaken and cerebral biopsies were obtained next to the lesion ("clinical penumbra") and from the corresponding contra-lateral side ("lesion's mirror"), to determine intracellular calcium by fluorescence signals of FURA-2 loaded cells. Results: Controls remained in hypotension and in a low flow state, while fluid resuscitation improved hemodynamic profile. There was a significant increase in intracellular calcium in the injured hemisphere in CT (1035±782 nM), compared to both HS (457±149 nM, p=0.028) and LR (392±178 nM, p=0.017), with no differences between HS and LR (p=0.38). Intracellular calcium at the contra-lateral, uninjured hemisphere, was 438±192 nM in CT, 510±196 nM in HS, and 311±51 nM in LR, with no significant differences between them. Conclusion: Both small volume hypertonic saline and large volume lactated Ringer's blunts calcium influx in early stages of TBI associated to hemorrhagic shock, suggesting a potential, early benefit, specially during immediate care and transport.

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PARACRINE REGULATION OF APOPTOSIS BY IL-1ß- AND IL-8-STIMULATED PMN: DIFFERENTIAL SUPPRESSION OF FASL AND TNF-α INDUCED APOPTOSIS. P.S. Grutkoski*, A. Ayala and H.H. Simms; Rhode Island Hospital and Brown University, Providence, RI 02903.

Purpose: We have shown that media conditioned by IL-1ß- and IL-8-stimulated PMN (CM-IL1ß and CM-IL8) suppress apoptosis (Ao) of fresh PMN by ~50% and 30%, respectively. While the active agents have yet to be identified, the purpose of this study was to begin to examine the mechanisms by which these CM exert their effects. Methods: PMN were incubated in CM ± FasL, TNF-α, or inhibitors for NF-κB, PI3K, p38MAPK, and MEK-1 for 8-12 hrs. and assayed for Ao. Fas and FasL were analyzed by western and FACS analyses of PMN incubated in CM for 1-8 hrs. Results: CM-IL1ß was able to block FasL induced Ao (%Ao: CM-IL1ß 20.7±3.2; CM+FasL 18.3±3.5; HBSS+FasL 70.5±4.7), but not TNF-α induced Ao (%Ao: 48.9±4.2). In contrast, CM-IL8 (%Ao: 38.2±1.9) blocked both FasL and TNF-α induced Ao (%Ao: 37.5±3.6 and 34.3±2.8, respectively). Inhibitors of PI3K, p38MAPK, or MEK-1 had no effect on the suppressive activity of either CM. Neither CM affected the total expression of Fas or FasL. Conclusions: We have shown that IL-1ß- and IL-8-stimulated PMN secrete factor(s) capable of suppressing spontaneous and FasL induced apoptosis. It appears that each cytokine promotes the secretion of distinct factors as only CM-IL8 can also suppress TNFα induced apoptosis. Finally, our data demonstrates that this suppression does not involve NF-κB, PI3K, MAPK cascades, or downregulation of cellular Fas or FasL.

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TNFR-I IS REQUIRED FOR HEAT STRESS INDUCTION OF CYTOPROTECTIVE HSP70 IN Mϕ J. Heimbach*, X. Meng*, L. Reznikov*, C. Calkins* B. Pomerantz*, C. Dinarello* A. Harken* (spon. R. McIntyre) University of Colorado Health Sciences Center. Box C-320. Denver, CO 80262.

Expression of heat shock proteins (hsp) is an adaptive response to cellular stress. The mechanism of induction of hsp70 by heat stress remains undefined. Although activation of TNF-α receptor I (TNFR-I) has been observed following osmotic stress or UV radiation, the role of TNFR-I in hsp70 expression is unknown. We **hypothesize** that the TNFR-I mediates heat stress induction of hsp70. **Methods**: Peritoneal Mϕ were isolated from wild type (C57), TNF-α knockout (KO), and TNFR (I or II) KO mice. Cells were incubated at 43° C for 30 min. Hsp70 protein induction was examined by immunoblotting. Hsp70 mRNA expression was examined using RT-PCR. **Results**: Heat stress induction of hsp70 protein expression is markedly decreased in the TNFR-I KO Mϕ , while TNF-α KO and TNFR-II KO Mϕ have normal induction of hsp70. Surprisingly, Hsp70 mRNA expression in TNFR-I KO Mϕ is comparable to wild-type.

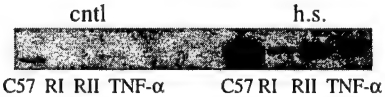

Figure 1. hsp70 production by immunoblotting

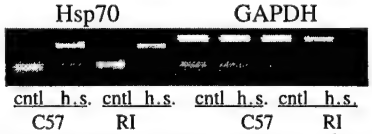

Figure 2. hsp70 gene expression by RT-PCR.

Conclusion: TNFR-I is required for heat stress induction of hsp70. However, heat stress induction of hsp70 via TNFR-I is independent of TNF-α. In addition, TNFR-I regulation of hsp70 expression appears to be at the level of protein translation. The results suggest a novel role for TNFR-I in the post-transcriptional regulation of hsp70 production.

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CEREBRAL VIABILITY AFTER GRADE IV HEMORRHAGE: IS IMMEDIATE FLUID RESUSCITATION NECESSARY? R. Miraliakbari*, P. Ng*, J. Philpott*, B.Taterosian*, V. Kim*, P. Brown*, M. Swanson*, Y. Sun*, R. Lust, and W. Chitwood Jr*. East Carolina University Brody School of Medicine, Departments of Surgery and Physiology, Greenville, NC 27858-4354.

Hemodilution compromises the benefits of crystalloid resuscitation by reducing oxygen delivery, which contradicts current trauma doctrine. To determine the effect on cerebral viability, this study compares blood, crystalloid, and no resuscitation in severe shock. Seventeen mechanically ventilated mongrel dogs were instrumented to measure hematocrits, blood gases, cerebral blood flow (CBF, ml/min/100g), resistance (CVR, mmHg/ml/min/100g), intracranial pressure (ICP, mmHg) and cerebral oxygen delivery (CDO_2, ml 0_2/100g/min). They were acutely bled to and maintained at a mean arterial blood pressure of 45 mmHg. Resuscitation was randomized to 3 strategies: 1) Bld (n=6, transfused shed blood), and 2) lactated ringers (LR) (n=6, received 3 x shed blood volume), 3) none (n=5, no resuscitation). The experiment was completed at 2.5 hrs after the initial hemorrhage. All animals sustained a ≥40% total blood volume loss. Final values are summarized below (mean ± SEM; *,p<0.05 vs. baseline; ^ p<0.05 vs. none):

	CBF	CVR	ICP	CDO_2
Base	26.0± 8.7	196.5±6.3	13.8±0.86	3.9±0.48
Bld	27.5±7.2	^*129±4.4	15.2±2.13	3.6±0.37
LR	^*47.3±1.7	*78.4±4.5	^*20.0±1.1	3.5±0.48
None	28.2±10.5	64.2±6.15	14.8±11.7	4.2±1.29

In the 3 groups, CDO_2 was preserved. However, in order to maintain CDO_2, LR resuscitation required a higher CBF and severe vasodilatation, creating a higher ICP. Blood resuscitation, in contrast, better maintained the dilator reserves. No resuscitation for a short time period proved no worse than crystalloid resuscitation. Within the context of cerebral viability, stable hypotension may be reasonably tolerated until blood resuscitation can be established.

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COX-1 INDUCTION AND IL1β EXPRESSION IN ALVEOLAR MACROPHAGES AFTER UNILATERAL CHEST TRAUMA. WJ Desselle*, JJ Greenhaw*, LL Trenthem*, TC Fabian, KG Proctor. Depts of Surgery & Physiology, Univ. Tenn. Health Science Center, Memphis

We have shown that unilateral trauma can activate a 2° inflammatory process and bilateral respiratory failure, which is attenuated with indomethacin, adenosine, or anti-oxidants. Inducible and constitutive cyclooxygenase (COX) or cytokine pathways in infiltrating neutrophils or alveolar macrophages (AMϕ) elaborate possible mediators of this process. The purposes of this study were: 1) to determine the early time course of COX isoforms and interleukin 1β (IL1β) expression in AMϕ; 2) to determine the functional significance of those changes. Methods: Cross-bred, anesthetized, ventilated swine were instrumented for cardiopulmonary function. A right unilateral injury was delivered by captive bolt gun, followed by 15% hemorrhage. After 1 hr, resuscitation consisted of thoracostomy, IV saline to hemodynamic endpoints, and O_2. AMϕ were isolated from bi-lateral broncho-alveolar lavages (BAL). COX-1, COX-2, and IL1β expression were determined with Western blots.

n=3-9	RL COX-1	RL IL1β	LLCOX-1	LLIL1β
0	6±3	1±1	4±1	2±1
40 min	89±36	2±2	87±35	1±0
60 min	188±89	2±1	125±51	1±0
80 min	124±57	2±1	74±19	3±1
100 min	124±73	10±7	54±21	5±2
120 min	89±27	28±18	11±4	32±20

No consistent changes in COX-2 were detected. COX-1 did not correlate with cardiopulmonary dysfunction, BAL protein, or pulmonary leukosequestration. Conclusions 1) following chest trauma, COX-1, not COX-2, is induced; 2) COX-1 peaks early, while IL-1β increases later, suggesting multiple mediators arise from AMϕ; 3) With bilateral responses after a unilateral stimulus, and with changes that are not correlated with injury severity, other mediators must be involved. Supported by Office of Naval Research

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ALVEOLAR MACROPHAGE TNF-α RELEASE IS ENHANCED FOLLOWING TRAUMA-HEMORRHAGE AND SEPSIS. D. Jarrar, J. F. Kuebler*, P. Wang, K.I. Bland*, and I.H. Chaudry. Brown Univ. and Rhode Island Hospital, Providence, RI 02903.

Despite significant advances in the treatment of critically ill patients, the adult respiratory distress syndrome (ARDS) is still a major cause of morbidity and mortality in the intensive care unit. However, it remains unknown whether local versus systemic proinflammatory cytokine release contributes to lung injury following trauma-hemorrhage and subsequent sepsis. To study this, male Sprague-Dawley rats (275-325g) underwent a laparotomy to induce soft tissue trauma. The animals were then bled to and maintained at a mean arterial pressure of 40 mmHg until 40% of the maximal bleedout (MB) volume was returned in the form of Ringer's lactate and were then resuscitated with 4 times the volume of MB with Ringer's lactate. At 20 h following the completion of fluid resuscitation, sepsis was induced by cecal ligation and puncture (CLP). Alveolar macrophages (Al. Mϕ) were harvested at 5 h following CLP, and assayed for TNF-α release. Serum levels (pg/ml) of IL-6 and TNF-α were also measured. Myeloperoxidase (MPO) activity in the lungs was determined enzymatically (U/mg protein). The results (means ± S.E. of 6-8 rats/group) were:

	Ser. IL-6	Ser. TNF-α	Al. Mϕ	MPO
Sham	ND	15±6	369±120	0.2±0.06
HCLP	1228±317*	120±70*	1057±314*	0.7±0.03*

(*p<0.05 vs Sham by Student's t-test; ND: non-detectable; HCLP: trauma-hemorrhage and CLP; Ser.: Serum.)

The results indicate that following HCLP, serum levels of proinflammatory cytokines as well as spontaneous Al. Mϕ TNF-α release were markedly elevated (p<0.05). Concomitantly, lung edema as assessed by wet/dry weight ratio and leukocyte activation was significantly increased following this "two-hit" injury. These results indicate that the systemic as well as local inflammatory response might contribute to lung injury and the subsequent development of ARDS. Therefore, therapies aimed at

attenuating the enhanced cytokine release might represent novel approaches for preventing ARDS and thus reducing the high morbidity and mortality following trauma-hemorrhage and subsequent sepsis. (Supported by NIH GM 39519).

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LETHAL SEPTIC SHOCK INCREASES MYOCARDIAL UCP-2 EXPRESSION COINCIDENT WITH MYOCARDIAL DYSFUNCTION

M. J. Roshon, J. A. Watts, J. A. Kline. Carolinas Medical Center, Charlotte, NC, 28232

Background: Myocardial depression with oxygen wasting occurs in-vivo during septic shock. We tested if myocardial UCP-2 mRNA expression would be increased with decreased cardiac work and efficiency after polymicrobial septic shock. Methods: Sepsis was initiated in ketamine/xylazine-anesthetized rats by cecal ligation and puncture (CLP). Tissues were freeze-clamped (-70 C) at 6 h and 12 h after septic insult for analysis of UCP-2 expression. Steady-state mRNA content was quantified by northern blot analysis with phosphoimaging to estimate band intensity. Additional hearts were removed after 12 h and perfused in working mode to measure work (mJ / min / g dry wt) and efficiency (CE= work/ oxygen consumption, %). Results: Myocardial UCP-2 mRNA expression was increased 4-fold (6 h) and 10-fold (12 h) compared to control hearts. Cardiac work (1.8 ± 0.2, shock vs 2.4 ± 0.1, control) and CE (9.9 ± 1.4 vs 13.5 ± 0.6; $p < 0.05$) were significantly decreased. Additional experiments determined that a 72 h mortality rate was 75% (9/12). Death occurred abruptly from 12-32 h with hypothermia (30 ± 1 °C). Conclusions: Lethal polymicrobial sepsis induces myocardial UCP-2 expression coincident with myocardial dysfunction with oxygen wasting immediately prior to the onset of rapid mortality in this model. UCP-2 expression could contribute to the transition from hyperdynamic, compensated shock to the uncompensated, hypodynamic shock that occurs during polymicrobial sepsis.

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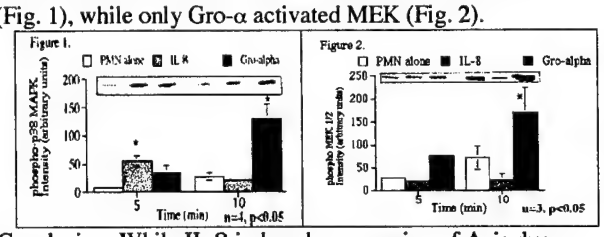
MECHANISMS OF PMN PERSISTENCE DURING INFLAMMATION: SUPPRESSION OF APOPTOSIS BY IL-8 AND GRO-α VIA DIVERSE SIGNALING MECHANISMS

A. Dunican*, S. Leuenroth*, P. Grutkoski*, A. Ayala, and H.H. Simms. Rhode Island Hospital, Providence, RI 02903.

Neutrophil (PMN) influx and persistence in the lung during systemic inflammation contributes to ARDS following traumatic shock. Suppression of PMN apoptosis (A_o) by chemokines including IL-8 and growth related oncogene-α (Gro-α) is central to this process. However, the intracellular signaling mechanisms through which these chemokines suppress A_o are unknown. Our hypothesis is that these chemokines suppress apoptosis through similar intracellular pathways. Methods: PMN were cultured in the presence of IL-8 or Gro-α and inhibitors to intracellular kinase pathways including MEK (PD098059) and p38 MAPK (SB202190). Western blot analysis was then performed to look at expression of phospho-p38 and phospho-MEK 1/2. Data is expressed as mean ± SEM and was analyzed by ANOVA. Results: IL-8-induced-suppression of PMN A_o (11±5%v. 81±5%-PMN alone) was blocked by SB202190 (73±6%) at 24 hr (n=4,$p<0.01$). Gro-α-induced suppression of A_o (15±4%) was also inhibited by p38 MAPK inhibitor and the MEK inhibitor, 69±5% and 54±5.6%, respectively (n=4, $p<0.01$). Western blot analysis demonstrated activation of p38 MAPK by IL-8 and Gro-α (Fig. 1), while only Gro-α activated MEK (Fig. 2).



Conclusion: While IL-8-induced-suppression of A_o is due primarily to activation of p38 MAPK, Gro-α mediates its effect through MEK and p38 MAPK. Understanding the differences in how chemokines signal the suppression of A_o may provide more specific therapeutic targets for the regulation of the inflammatory response.

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THE DISSOCIATION BETWEEN UPREGULATED ENDOTHELINS AND HEMODYNAMIC RESPONSES DURING POLYMICROBIAL SEPSIS.

DA Ornan*, IH Chaudry, and P Wang. Brown University School of Medicine and Rhode Island Hospital, Providence, Rhode Island 02903

Although studies have suggested endothelins (ETs) contribute to the development of endotoxic shock, little is known about the role of ETs in the transition from the hyperdynamic phase to the hypodynamic phase during the progression of sepsis. To study this, male adult rats were subjected to sepsis by cecal ligation and puncture (CLP) followed by fluid resuscitation. Plasma levels of ET-1 and ET-2 were measured by RIA at 2, 5, 10, and 20 h following CLP or sham operation (n=7-10/group). Tissue levels of ET-1 and ET-2 were determined in the heart, lungs, small intestine, and spleen at 5 h following CLP or sham operation (n=5-7/group). In addition, preproendothelin-1 gene expression was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) at 5 h in the heart, lungs, small intestine, spleen, and liver (n=3-5/group).

The results indicate that plasma levels of ET-1 and ET-2 did not increase significantly as compared to shams at 2 and 20 h after CLP, but increased by 123% and 70% ($p<0.05$) at 5 and 10 h after CLP, respectively. While there were no significant increases in tissue levels of ET-1 and ET-2 at 5 h post-CLP, RT-PCR analysis indicates a significant increase in preproendothelin-1 gene expression at 5 h after CLP in the heart, spleen, and liver (the ratio of target to housekeeping gene increased by 33-100%, $p<0.05$). There were no significant increases in gene expression in the lungs or small intestine. These results suggest that the heart, spleen, and liver are important ET-producing organs during the early stage of sepsis. The lack of significant increases in tissue levels of ETs suggests that the newly converted protein may be quickly transferred to the bloodstream. Since the hyperdynamic phase of sepsis occurs at 2-10 h and the hypodynamic phase occurs at 20 h after CLP, the increased

plasma levels of ETs at 5 and 10 h suggest that mediators other than ETs are responsible for producing the biphasic hemodynamic alterations during sepsis.

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IMMEDIATE EARLY GENES (IEG) AND TRANSCRIPTION FACTORS IN LIVER OF RATS PRECONDITIONED WITH CURCUMIN AND PICROLIV DURING HEMORRHAGIC SHOCK AND RESUSCITATION. G.S. Sidhu*, S.Sundar*, P.Seth* and R.K.Maheshwari*, (Spon:F. Rollwagen). Uniformed Services University of Health Sciences, Bethesda, MD 20814.

Initiation of cytokines cascades, and activating intracellular signal transduction pathways follow the transactivation of transcription factors such as AP-1, NF-kB, HSF-1 and HIF-1. The gene transcription and signal transduction are regulated by transcription factors and are central to processing and regulatory cellular homeostasis during hemorrhagic shock and resuscitation. The expression of IEG such as c-jun and c-fos, and the activation of AP-1 and NF-kB were examined in liver of rats. Curcumin (diferuloylmethane), a major component of the food flavor turmeric was isolated from the rhizomes of *Curcuma longa* and picroliv was derived from the rhizomes of *Picrorrhiza kurroa*. Both curcumin and picroliv increased the survival of rats during hemorrhage and resuscitation. Curcumin (40mg/kg) and picroliv (12mg/kg) were fed to animals once daily by oral gavage for 7days prior to experimental procedure. Shock was initiated in anesthetized rats by bleeding of 30ml /Kg body weight from femoral artery. After 1 hour, the rats were resuscitated with 2X volume of lactate Ringer's solution. The animals were sacrificed 2 h post-resuscitation. Curcumin and picroliv decreased the expression of IEG induced in the liver by hemorrhage/resuscitation shock as revealed by Western analysis. EMSA analysis showed that both the AP1 and NF-kB were transactivated in liver of hemorrhage controls compared to sham controls. Curcumin pretreatment inhibited the transactivation of both AP-1 and NF-kB, however, NF-kB remains unaltered by picroliv, suggesting that curcumin and picroliv protects against hemorrhage and resuscitation by regulating the gene transcription involved in cell dysfunction. (Supported by Office of Naval research: Grant # G174HV).

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GENETIC AND GENDER COMPONENTS IN THE EXPRESSION OF TUMOR NECROSIS FACTOR-α IN MICE DURING ENDOTOXEMIA. F. D. Stewart,* W. B. Fulton,* R.H. Reeves,* C. N. Paidas,* A. De Maio. Johns Hopkins School of Medicine, Baltimore, MD 21205.

The purpose of this study was to evaluate possible genetic and gender differences in the expression of tumor necrosis factor-α (TNF-α) in a model of endotoxemia in mice. Females (n=10) and males (n=10) of two inbred strains of mice, C57Bl/6J(B6) and A/J, which are known to have marked differences in the inflammatory response, were maintained in a pathogen free environment. Males from the F_1 generation between female A/J and male B6 (AXB, n=13) or female B6 and male A/J (BXA, n=10) were also used in this study. Mice were injected intraperitoneally with *E.coli* lipopolysaccharide (LPS, 15 mg/kg). TNF-α plasma levels were determined by a commercial ELISA in blood samples collected at 1.5 hours after the injection, the timepoint when both strains of mice showed maximal levels of this cytokine. Our results show that male A/J mice had a 5.3 fold higher plasma level of TNF-α than male B6 mice. TNF-α levels in A/J females (9537 pg/ml) were 2.8 fold higher than A/J males (3366 pg/ml, $p<0.018$). Also, B6 females (2987 pg/ml) showed a 4.2 fold excess over B6 males (630 pg/ml, $p<0.001$). F_1 males (AXB or BXA) showed a TNF-α phenotype similar to B6 males, with levels 5.6 fold lower than A/J males. Analysis of the F1 generation demonstrates that the mode of inheritance of the TNF-α phenotype is not sex-linked. These results indicate that the expression of TNF-α during endotoxemia is genetically and gender-specifically influenced. Supported by NIH grant GM 20026 and the Robert Garrett Foundation.

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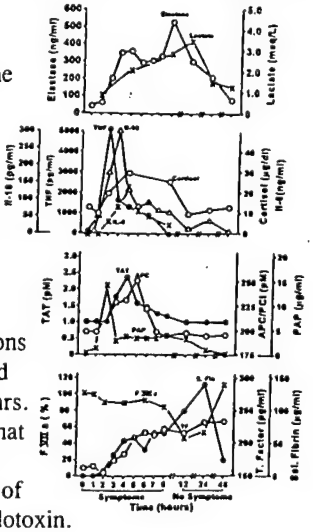
TWO STAGE RESPONSE TO ENDOTOXIN INFUSION INTO NORMAL HUMAN SUBJECTS. FB Taylor, Jr, PA Haddad, E Hack, GT Kinasewitz, ACK Chang, GT Peer*, JH Morrissey, A Li, RC Allen. OMRF, OKC, OK; OUHSC, OKC, OK; Emory University School of Medicine, Atlanta, GA; Central Laboratory of the Netherlands, Amsterdam.

Objective: The objective was to characterize both the immediate (1st stage) and delayed (2nd stage) response of normal human subjects to an IV bolus of endotoxin using phagocyte luminescence and molecular markers of hemostatic, phagocyte and endothelial cell perturbation.

Measurements: The response of eight healthy subjects to endotoxin was studied using luminescence measurements of phagocyte oxidase, oxidase-myeloperoxidase, opsonin receptor expression, and clinical chemical and molecular markers of hemostatic, inflammatory, and endothelial cell perturbation.

Results: Response to IV bolus endotoxin includes two stages. The first symptomatic stage (T-0 to T+8 hours) included a sharp rise in phagocyte respiratory activity and receptor expression and appearance of molecular markers of phagocyte, inflammatory, hemostatic system activation and endothelial cell injury. The second asymptomatic stage (T+8 to 48 hours) included a second peak of oxidase activity at T+24 hours in the face of a declining lactate and opsonin receptor expression. This coincided with a second high peak of soluble fibrin, sustained elevated concentrations of soluble thrombomodulin and a fall in fVIIa at T+12 to 24 hrs.



Conclusions: We conclude that this second stage represents a recovery or reperfusion phase of a compensated response to endotoxin.

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CHARACTERIZATION OF LOCAL AND SYSTEMIC CYTOKINE RESPONSES DURING ACUTE INFLAMMATION IN HUMANS.

FA. Rivera-Chavez, R. Munford, and G. O'Keefe. Univ. of Texas Southwestern Med. Ctr. Dallas, TX 75235-9160 **Background:** The local and systemic host responses to injury and infection involve complex interactions among a variety of mediators. These interactions have traditionally been considered to produce a "pro-inflammatory" state. However, the net impact of pro- and anti-inflammatory mediators in local vs. systemic compartments is uncertain. We have used appendicitis as a model of moderately severe localized infection and compared mediator concentrations in the local (peritoneal fluid,PF) and systemic (plasma,PL) compartments.

Methods: PL and PF were obtained from patients with appendicitis and from non-infected control patients. PF and PL cytokine concentrations were measured by ELISA. To assess the ability of the PL or PF to alter cell responses to a bacterial agonist, the fluids were incubated with cultured human monocytes (THP-1) and LPS (0.05 – 1 ng/ml) for 2h at 37°C and supernatant IL-8 was then measured.

Results: IL-6 (1104 ± 707 pg/ml) and IL-8 (200 ±158 pg/ml) were found in the PF of patients with appendicitis but not in control PF.. In contrast, IL-10 (175 ± 96 pg/ml) and IL-4 (183 ± 13 pg/ml) were found in the PL of patients with appendicitis but not in control PL, whereas IL-6 and IL-8 were not found in PL. When compared with the corresponding samples from controls, PF from appendicitis patients enhanced, while PL decreased, LPS-induced IL-8 release by THP-1 cells.

Conclusions: Acute appendicitis is associated with a pro-inflammatory local (PF) response while the systemic (PL) response seems to be anti-inflammatory.

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SAFETY AND EFFICACY OF HYPERTONIC SALINE DEXTRAN IN PEDIATRIC PATIENTS SUBMITTED TO CARDIAC SURGERY WITH CARDIOPULMONARY BYPASS. R Rocha e Silva*, LF Canêo*, DD Lourenço-Filho*, SM Franchi*, CMC Afiune*, MB Jatene*, M Barbero-Marcial*, M Rocha e Silva. Heart Institute-InCor, Univ. São Paulo Medical School, SP 05403-000, Brazil.

Hypertonic saline dextran (HSD: 7,5% NaCl + 6% Dextran-70) has been used in adults (but not in children) submitted to open heart surgery inducing negative fluid balance and reducing blood/derivative requirements. This study determined the safety and efficacy of HSD in 30 children (age: 2-11 years, body weight 12-35 kg) undergoing open heart surgery with cardiopulmonary bypass (CPB) for atrial septal defect correction. Associated procedures were: 10 tricuspid anuloplasty and 1 infundibular pulmonary stenosis correction. Operations were performed with bloodless priming of extracorporeal circulation under moderate hypothermia. Children were divided in 6 groups of 5 subjects. HSD in each group was incrementally dosed, 0, 0.1, 0.5, 1.0, 2.0 and 4,0 ml/kg, given 5 min. before the beginning of each CPB. For statistical analysis patients were divided in **LOW** (0-1ml/kg) versus **HIGH** (2-4ml/kg) dose of HSD. Blood loss, 24hr fluid balance, and blood/derivative requirements were compared. No differences occurred in the amount of bleeding (T test). Fluid balance and % of patients receiving blood/derivatives were significantly lower in the **HIGH** dose group (T test, and Fisher exact test, respectively). No complications were related to HSD in this study.

DOSE OF HSD ▶	LOW	HIGH	p
Intra-surgical bleeding (ml/kg)	7.0±3.2	5.2±1.3	0.106
Post-surgical bleeding (ml/kg)	6.1±3.1	5.1±2.6	0.409
Fluid balance (ml/kg)	6.9±22.7	-15.6±14.9	**0.008**
Blood/derivative requirements	45%	0%	**0.013**

Thus, HSD is safe in the pediatric population submitted to CPB, does not lead to coagulopathies, produces negative fluid balance and reduces the need for blood and derivatives.

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PREVENTION OF MULTIPLE ORGAN FAILURE (MOF) SECONDARY TO SEVERE ACUTE PANCREATITIS (SAP) WITH CONTINUOUS HEMODIAFILTRATION (CHDF) AND SELECTIVE DIGESTIVE DECONTAMINATION (SDD)

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DEPT EMERG. & CCM, CHIBA, UNIV. SCH. MED, CHIBA, JAPAN, 260-8677

SAP often develops sepsis and MOF. It has been claimed that MOF secondary to SAP is caused by the overactivation of humoral mediator network and that septic complication of SAP is often resulted from bacterial translocation (BT). On the other hand, we reported that CHDF could remove variety of humoral mediators from the blood stream of a patient and that SDD was effective in the prophylaxis of BT. Therefore, the present study was undertaken to investigate the efficacy of CHDF and SDD in the prevention of MOF secondary to SAP. Thirty patients diagnosed to have SAP entered to the study. CHDF was performed regardless of their renal function aiming at the removal of humoral mediators. SDD was given to all patients. The changes in the blood level of humoral mediators such as interleukin-6 (IL-6) with CHDF were studied. Also studied was the incidence of septic complication and MOF. Twenty-eight patients of 30 (93.3%) survived. Blood level of IL-6 significantly elevated in SAP patients, especially in SAP patients with organ failure at the entry to the study, compared to normal controls. However, IL-6 blood level significantly decreased with CHDF and the degree of decrease in IL-6 significantly and positively correlated with the degree of improvement in organ functions such as respiratory function and tissue oxygen metabolism. SDD significantly decreased the incidence of septic complication compared to the another set of SAP patients who did not receive SDD (14% vs 56%, $p<0.05$). Thus we conclude that CHDF and SDD are very effective prophylactic measures against MOF secondary to SAP.

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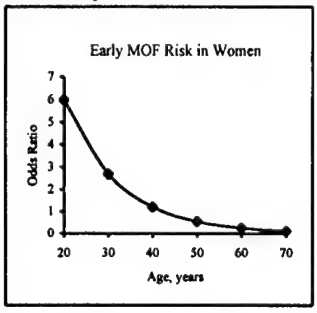
FEMALE GENDER IS A RISK FACTOR FOR EARLY POSTINJURY MULTIPLE ORGAN FAILURE. P. Offner, E. Moore, W. Biffl, Denver Health Medical Center, Denver, CO 80204

Animal studies have documented immunosuppression in males following trauma/hemorrhage. We reported that male

gender is a significant risk factor for major infections following severe injury. Infections have been associated with late postinjury multiple organ failure (MOF). The **purpose** of this study was to investigate the effect of gender on the frequency and pattern (early vs late) of postinjury MOF. **Methods**: Patients admitted to our Level I trauma center with ISS >15, age > 15 and survival > 48 hours were prospectively identified and entered into our MOF database. MOF was determined by the Denver MOF scoring system. MOF was considered early if present by ICU day 3, and late if diagnosed thereafter. Using logistic regression analysis, gender was evaluated as an independent risk factor for MOF. Similar analyses were performed for early versus late MOF. **Results**: 545 patients were prospectively enrolled between 1993 and 1998. 135 (25%) were female and 410 (75%) were male. MOF occurred in 87 (16%) patients; early in 34 (39%) and late in 53 (61%) patients. Multivariate analysis demonstrated that female gender is an independent risk factor for early but not late MOF. There



was a significant interaction between age and gender with women ≤ 45 years having a 3-fold greater risk of early MOF (p=.027). Risk progressively decreased with advancing age. **Conclusions**: Female gender is associated with dramatically increased risk of early postinjury MOF. This may reflect lack of immune suppression noted in males following trauma, potentially exacerbating dysfunctional hyperinflammation culminating in MOF. The restriction of this effect to women less than 45 years suggests a hormonal mechanism.

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HYPOXIA INHIBITS iNOS EXPRESSION IN ENDOTHELIAL CELLS. H. Bitterman, M.A. Rahat*, A. Kinarty* and N. Lahat*, Carmel Medical Center, Faculty of Medicine, Technion, Haifa 34362, Israel.

Hypoxia and reoxygenation (H/R) are key components of ischemia and reperfusion. We studied the effects of H/R on NOSes and NO production in the mouse endothelioma cell line bEND3. Cells were subjected to H/R by exposure to either hypoxic (<1% O2, 5% CO2, 95% N2) or normoxic (21% O2, 5% CO2, 74% N2) gas mixture for 2 or 24 hours, followed by re-oxygenation with room air for 2 or 24 hours with or without stimulation with 100 U/ml interferon-γ (IFNγ), 1 μg/ml LPS, or their combination. In normoxic conditions 24 hours of hypoxia with both IFNγ and LPS were required to induce endothelial iNOS expression (12-fold increase over controls, $p<0.001$). Hypoxia markedly attenuated the increase in iNOS expression ($p<0.001$) and prolonged re-oxygenation (24 hrs) restored it to normoxic levels. Expression of ecNOS did not change by either H/R or exogenous stimuli. Basal values of nitrites and nitrates (40±9 μM) were constitutively secreted by bEND3 cells, and increased to 95±20 μM ($p<0.01$) after 48 hours of normoxic incubation and stimulation with IFNγ and LPS. Accumulation of NO products was significantly attenuated by prolonged H/R (61±13 μM, $p<0.05$). Our data indicate that endothelial cells can be induced by immune/inflammatory signals to express iNOS. In contrast to published data in macrophages hypoxia attenuates the expression of iNOS in endothelial cells. Furthermore, H/R did not affect the expression of endothelial ecNOS suggesting that hypoxia alone does not explain previous reports of decreased ecNOS activity in endothelial cells after ischemia and reperfusion.

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NITRIC OXIDE PRE-TREATMENT PROTECTS AGAINST PEROXYNITRITE-INDUCED ENTEROCYTE APOPTOSIS
DA Potoka*, JS Upperman*, and HR Ford
Children's Hospital of Pittsburgh, Pittsburgh, PA 15213

Purpose: Nitric oxide (NO) may have both beneficial and detrimental effects on intestinal physiology during sepsis or shock. Constitutive low levels of NO may be protective by regulating mucosal blood flow and permeability. However, sustained overproduction of NO can be harmful by combining with superoxide to form the toxic metabolite, peroxynitrite ($ONOO^-$), which can induce enterocyte apoptosis and lead to gut barrier failure. Furthermore, NO itself can inhibit apoptosis in some systems. We sought to determine if NO pre-treatment protects against $ONOO^-$-induced apoptosis in enterocytes. **Methods:** Rat intestinal epithelial cells (IEC-6) were grown to confluence. Cells were treated with the NO donor SNAP (0.0625, 0.125, 0.25, or 0.5 mM) for 12 hr. After SNAP treatment, the media was removed and media nitrite concentration (an end-product of NO) was measured using the Griess reaction. Cells were then treated with 50 μM $ONOO^-$ or decomposed $ONOO^-$ (control) for 60 min followed by a 24 hr recovery in media. Apoptosis was then assayed using flow cytometry with annexin V-FITC and propidium iodide staining. **Results:** SNAP alone, up to 0.5 mM, had no effect on baseline IEC-6 apoptosis. Increasing the concentration of SNAP exposed IEC-6 cells to correspondingly more NO as measured by nitrite production (8.2 μM for 0.0625 mM SNAP vs. 85.2 μM for 0.5 mM SNAP). SNAP pre-treatment partially inhibited $ONOO^-$-induced IEC-6 apoptosis to a similar degree for each concentration (low and high SNAP dose data shown in Table).

	APOPTOSIS (%)		
	No SNAP	0.0625 mM SNAP	0.5 mM SNAP
50 μM Control	4.2 ± 2.2	4.6 ± 3.3	7.2 ± 6.1
50 μM ONOO	19.7 ± 5.9	12.8 ± 5.7†	12.4 ± 6.9†

†$p<0.05$ vs No SNAP by Fisher's least significant difference

Conclusion: NO pre-treatment partially protects against $ONOO^-$-induced enterocyte apoptosis. This suggests a mucosal protective role for NO at the cellular level.

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THE ABSENCE OF eNOS INCREASES MORTALITY AFTER HEMORRHAGIC SHOCK.
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Short-term studies using animal models of hemorrhagic shock have demonstrated increased organ injury using nonselective nitric oxide synthase (NOS)

inhibitors and decreased organ injury using inhibitors selective for iNOS when compared with controls. Because most studies to date have utilized pharmacologic manipulation using nonspecific inhibitors, it is not clear how endothelial constitutive NOS (eNOS) and inducible NOS (iNOS) each contributes to survival and mortality in hemorrhagic shock. We hypothesized that the absence of eNOS would adversely affect survival, whereas the absence of iNOS would improve survival after hemorrhagic shock. To test our hypothesis, mice deficient in the eNOS gene [eNOS (-/-)] and in the iNOS gene [iNOS (-/-)] were bled to a mean arterial blood pressure of 25 mmHg for 2.5 hours and resuscitated with return of shed blood and crystalloid. Survival at 24 hours was compared against wild type (WT) control mice. Results: All sham instrumented animals (n=4 per group) were alive at 24 hours (100% survival). Of the shocked animals, 4 out of 6 WT as well as iNOS (-/-) mice survived 24 hours (67% survival per group). However, only 2 out of 6 eNOS (-/-) mice survived 24 hours (33%). These results suggest that 24-hour survival is comparable between iNOS deficient mice and WT controls, but that the absence of eNOS increases mortality after hemorrhagic shock.

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Effects of *n*-acetylcysteine on ischemic brain injury

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[1]Institute of Pharmacology, [2]Department of Biomorphology, University of Messina, Italy;

Nitric oxide (NO), peroxynitrite, formed from NO and superoxide anion, poly (ADP-ribole) synthetase have been implicated as mediators of neuronal damage following focal ischemia. Here we have investigated the effects of *n*-acetylcysteine (NAC) treatment in Mongolian gerbils subjected to cerebral ischemia. Treatment of gerbils with NAC (20 mg/kg 30 minutes before reperfusion and 1, 2 and 6 hours after reperfusion) reduced the formation of post-ischemic brain oedema, evaluated by water content. NAC also attenuated the increase in the brain levels of malondialdehyde (MDA) and the increase in the hippocampus of myeloperoxidase (MPO) caused by cerebral ischemia. Positive staining for nitrotyrosine was found in the hippocampus from in Mongolian gerbils subjected to cerebral ischemia. Hippocampus tissue sections from Mongolian gerbils subjected to cerebral ischemia also showed positive staining for poly (ADP-ribose) synthetase (PARS). The degree of staining for nitrotyrosine and for PARS were markedly reduced in tissue sections obtained from animals that received NAC. NAC treatment increased survival and reduced hyperactivity linked to neurodegeneration induced by cerebral ischemia and reperfusion. Histological observations of the pyramidal layer of CA1 showed a reduction of neuronal loss in animals that received NAC. These results show that NAC improves brain injury induced by transient cerebral ischemia.

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NITRIC OXIDE SYNTHASE INHIBITOR AMELIORATES ORAL TOTAL PARENTERAL NUTRITION-INDUCED BARRIER DYSFUNCTION L.-W. Chen, C.-M. Hsu* and J.-S. Chen*, Veterans General Hospital-Kaohsiung and National Sun Yat-Sen University, Kaohsiung, Taiwan.

The oral administration of total parenteral nutrition (TPN) solution has been shown to promote bacterial translocation (BT) and increase the intestinal permeability, the role of NO in the nutrition-induced loss of mucosal barrier function remains unclear. Rats were divided into four groups. Group I (control group) were fed with rat chow, Group II received TPN solution orally. Group III and IV received TPN solution supplemented with NOS inhibitors. On day 9, fluorescein isothiocyanate-dextran (FITC-dextran) was injected into the intestinal lumen. After 30 min, blood samples were taken from portal vein for plasma FITC-dextran assay. Homogenates of small intestine were used for NOS activity measurement. The plasma level of FITC-dextran showed a significant increase ($P < 0.05$) in rats fed with oral-TPN. Supplement with NOS inhibitors significantly decreased the intestinal permeability in Group III and IV compared with Group II. Similarly, the total NOS activities showed a significant 2-fold increase ($P < 0.05$) in Group II and NOS inhibitors decreased the elevated NOS activity. These data suggest that oral-TPN feeding for 9 days leads to an increase in permeability to dextran and the total NOS activity of small intestine, and both induction of the intestinal permeability and NOS activity were inhibited by treatment with NOS inhibitors. Addition of S-methylisothiourea (SMT), an *i*NOS selective inhibitor, inhibited 66 % of the induced *i*NOS activity ($P < 0.05$) and reduced 74 % of the diet-induced increase in intestinal permeability ($P < 0.05$) in Group II. The induction of *i*NOS is an important mediator for intestinal barrier dysfunction. Administration of SMT, specifically decreases *i*NOS activity, is useful in the prevention of diet-induced barrier failure.

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ACTIN CYTOSKELETON AND ENDOTHELIAL CELL RESPONSE TO OSMOTIC STRESS

S. Arbabi, M.R. Rosengart*, I. Garcia*, and R.V. Maier, University of Washington, Seattle WA 98195.

The endothelium plays an important role in the host response to a variety of environmental stimuli. Endothelial response to osmotic stress, such as hypertonic saline, is partly mediated via activation of p38 and ERK, two members of the mitogen-activated protein kinase (MAPK) family. The mechanism of osmotic stress activation of the MAPK cascade is ill defined. Clearly, the cytoskeleton changes following cytoplasmic shrinkage in response to osmotic stress. Thus, we hypothesized that the actin cytoskeleton is essential in translating osmotic stress-induced morphological alterations into p38 and ERK activation. **Methods:** Human umbilical vein endothelial cells were

subjected to osmotic stress with varying concentrations of sodium chloride (NaCl), 40-100 mM. Some of the cells were pretreated with 2 μM of Cytochalasin D (CD), an agent that disrupts actin polymerization. Total cellular protein was extracted at different time points and subjected to Western blot analysis using dual phospho-specific antibodies that only recognize the active species of p38 or ERK. **Results**: CD pretreatment disrupted the actin cytoskeleton, collapsed the endothelial cell body, and reversed cell spreading. In the non-pretreatment group, osmotic stress induced maximal p38 and ERK activation in a dose dependent manner with maximal activation within 60 minutes and a return to baseline in 4 hours. The osmotic-induced ERK activation was inhibited in the presence of CD. However, p38 activation was not inhibited and actually demonstrated an enhanced response. **Conclusion:** The integrity of actin cytoskeleton is essential for osmotic-induced ERK activation, however, such is not the case for p38 activation. Similar to adherence-induced activation, osmotic stress may use the interaction of actin cytoskeleton with focal adhesion kinase related proteins to activate ERK. It appears that the mechanism for osmotic-induced p38 activation is different and has yet to be identified.

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VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXERTS BENEFICIAL EFFECTS IN TRAUMATIC SHOCK VIA PRESERVATION OF VASCULAR ENDOTHELIAL FUNCTION.

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Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

VEGF is an endothelium-specific secreted protein that induces vasodilation and increases endothelial release of nitric oxide (NO). Loss of endothelium-derived NO is an integral part of the initiation and maintenance of the inflammatory process similar to that occurring in traumatic shock, and is considered responsible for much of the trauma-induced microvascular injury. Therefore, we investigated the effect of VEGF in pentobarbital-anesthetized rats subjected to Noble-Collip drum trauma. Trauma rats developed a shock state characterized by marked hypotension and a 93% mortality rate with a mean survival time of 108 ± 10 min in 14 rats. Following trauma, a significant degree of endothelial dysfunction, and a markedly elevated intestinal myeloperoxidase activity were also observed. Intravenous administration of 125 μg/kg VEGF 18 hours pre-trauma, increased survival rate to 67% ($p<0.01$), and prolonged survival time to 252 ± 24 min in 12 rats ($p<0.01$). VEGF also significantly preserved the endothelium-dependent release of NO. Our results indicate that endothelial dysfunction, with its accompanying loss of NO, plays an important role in tissue injury associated with trauma, and that preservation of endogenous endothelium-derived NO is beneficial in traumatic shock. The mechanisms of the protective effect of VEGF in trauma involves preservation of eNOS function and diminished neutrophil accumulation resulting in reduced neutrophil-mediated tissue injury.

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Shock Induces Bone Marrow Injury and a Migration of Hematopoietic Precursors to Remote Organs which is Partially Mediated Through Mesenteric Lymph.

DJ Anjaria*, P Rameshwar, D Xu, CA Adams*, RM Forsythe*, JT Sambol*, CJ Hauser, EA Deitch, DH Livingston. New Jersey Medical School, Newark, NJ 07103

Hypothesis: Shock induced bone marrow (BM) injury is mediated through mesenteric lymph, and therefore should be ameliorated with pre-shock lymph ligation.

Methods: Male Sprague-Dawley rats were divided into 3 groups: unmanipulated controls (C), shock (S), or shock with lymph ligation (SL). Shocked animals (MAP=30mmHg for 90 min) underwent laparotomy +/- mesenteric lymph ligation. Animals were resuscitated with blood and fluid and sacrificed at 3 hours. Mononuclear cells obtained from BM, peripheral blood, lung, liver and spleen and cultured for CFU-GM, CFU-E (late hematopoietic precursors), and cobblestone area forming cells (CAFC – a hematopoietic stem cell assay).

Results: BM cellularity decreased 52% compared to C after shock. SL restored cellularity to C values. Data for BM (per femur) and lung (per plate) precursors are shown below.

Group	BM CFU-GM	BM CFU-E	BM CAFC	Lung CFU-GM	Lung CFU-E	Lung CAFC
C	250	240	36	5	1	2
S	125	110	18	37	21	12
SL	400	410	30	21	24	3

A similar pattern to lung was observed in liver, spleen and peripheral blood. Shock clearly induces a shift of BM precursors to distant organs. SL maintained BM precursors numbers and decreased the number of CAFC (stem cells) found in the periphery to C values

Conclusion: Shock induces the loss of early and late precursors from BM which appears to be mediated by mesenteric lymph. Lymph ligation influences early (CAFC) but not late (CFU-GM and CFU-E) hematopoietic precursors migrating to the periphery. As lymph ligation is associated with an improvement in post-shock pulmonary function it remains to be determined whether these BM stem cells contribute to the organ dysfunction observed following shock and trauma.

AUTHOR-ABSTRACT INDEX

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1. Ohtake Y, Clemens MG: Interrelationship between hepatic ureagenesis and gluconeogenesis in early sepsis. *Am J Physiol* 260:E453–E458, 1991.

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2. Chaudry IH, Ayala A: *Immunological Aspects of Hemorrhage.* Austin TX: RG Landes Company, 1992, pp 1–132.

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3. Dunn DL: Intra-abdominal sepsis. In Jurst JM (ed): *Common Problems in Trauma.* Chicago IL: Yearbook Medical Publishers, 1987, pp 392–398.

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